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Development of the posterior crossvein in crossveinless-like strains of Drosophila melanogaster can be influenced by high temperature shocks at specific times in pupal development.

Three sensitive periods were detected in age response studies on Ona X, Ona II, 5-hi III; crossvein restoration at 14 hours in pupal development, a crossvein limiting response at 22 hours in pupal development and a reduction in penetrance at 28 hours in pupal development. A further characterization of these responses revealed that at 22 hours there also exists a rapid crossvein restoration which appears to be identical with the response at 14 hours, and another restoration following long durations of treatment at 22 hours. At 28 hours an additional crossvein limiting response was observed.

Temperature coefficients have been calculated for all the responses, except those at 28 hours, and these coefficients indicate that the basis of the various responses are due to conformational changes in protein following temperature denaturation.

Preliminary genetic studies of the responses showed a profound effect of the major gene, and this gene was localized to 48.1 on the genetic map of the third chromosome.

The data have been interpreted as being consistent with affecting either a control mechanism involved in the development of the posterior crossvein, or a reaction sequence involved in the development of the posterior crossvein.

THE EFFECT OF TEMPERATURE ON CROSSVEIN FORMATION IN CROSSVEINLESS-LIKE STRAINS OF *DROSOPHILA MELANOGASTER* (MEIGEN)

by

STEVEN RISLEY THOMPSON

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APPROVED:

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Associate Professor of Zoology
In Charge of Major

[redacted]

Chairman of Department of Zoology

[redacted]

Dean of Graduate School

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THE EFFECT OF TEMPERATURE ON CROSSVEIN FORMATION IN CROSSVEINLESS-LIKE STRAINS OF *DROSOPHILA MELANOGASTER* (MEIGEN)

INTRODUCTION

In recent years there has been developed a macro-molecular theory of primary gene action, based primarily on studies with microorganisms. The principles derived from this theory, i.e., the information coded within a specific sequence of nucleotides in the DNA molecule is transcribed into a specific RNA and this specificity is translated into a specific polypeptide, appear to have general significance in higher organisms. It is yet necessary to ascertain in what fashion the activities of the specific polypeptides and their interactions are functioning in the formation of the complex phenotype of higher organisms. For, it is apparent that the development of morphological characters must involve mechanisms which control differential gene activity during the sequence of events which lead to the formation of a particular phenotype.

The posterior crossvein of *Drosophila melanogaster* provides appropriate material for studying the mechanisms which control the development of morphological characters. This structure has been shown to be an extremely sensitive developmental system which is affected by genetic variation at several loci, and which is susceptible to environmental stress, such as temperature shock,

at different times in development.

In addition to mutants of large effect, such as crossveinless (cv), a number of investigators have shown that it is possible to select for polygenes (genes of small individual effect) which disrupt the normal phenotype and produce crossvein defects (Bateman, 1959; Milkman, 1960a, b, 1964, 1965a; Mohler, 1965b; and Waddington, 1953). Methods have been developed which can be used to limit the number of varying genes and to specify particular combinations of these genes (Milkman, 1965b; Mohler, 1965b). Mohler (1965b, 1966) has employed these techniques to study the genetics of some of the crossveinless-like strains.

The response of the posterior crossvein to temperature shock was first demonstrated by Goldschmidt (1935). He showed that heat shock can cause the appearance of phenotypes which copy those caused by mutants such as crossveinless. Milkman (1961, 1962, 1963) demonstrated that the phenocopy response to high temperature in wild-type Drosophila can be interpreted as due to a series of changes in the tertiary structure of a protein necessary for the formation of complete crossveins. The response of crossveinless-like lines to temperature shock have been studied by Milkman (1962, 1964, 1966) and by Mohler (1965a). They have demonstrated that some of these selected lines contain genes which show increased susceptibility to heat shock while others do not,

and that the heterozygotes from crosses of susceptible crossveinless-like strains with a wild-type are also more sensitive to heat shock (Milkman, 1961; Mohler, 1965a). These observations suggest that the temperature shock is acting upon the same functional system as some of the genes whose action it copies.

Phenocopy experiments with wild-type Drosophila have demonstrated a crossvein limiting response, recognized by the production of crossvein defects (Milkman, 1961, 1962, 1963). However, phenocopy experiments with crossveinless-like strains have shown, in addition to a crossvein limiting response, crossvein restoring responses (Milkman, 1966; Mohler, 1965a). The sensitive periods of these two opposing responses are well in advance of the visible morphological effects of crossvein formation (Mohler and Swedberg, 1964; Wong, 1966a). Thus, it may be possible that some of the components recognized in phenocopy experiments are involved in the normal control mechanisms of the wild-type, and that temperature shock can be employed to recognize the regulators and separate them on the basis of the kinetics due to temperature denaturation. Indeed, Sadler and Novick (1965) have demonstrated that the repressor molecule, in the β-galactosidase control system of Escherichia coli, is at least in part protein and has similar kinetics as those demonstrated by Milkman (1961, 1962, 1963) in the crossveinless phenocopy response.

This thesis is an investigation of the types and the nature of the responses to temperature shock in a crossveinless-like strain of Drosophila melanogaster. It will be shown that there are at least three responses to temperature shock, two involved in crossvein restoration and a crossvein limiting response. The kinetics of these responses will be demonstrated to be consistent with conformational changes in protein, and the relationship of the responses to control mechanisms will be discussed. Further, preliminary studies show a profound effect of "major gene" substitution on the responses to temperature shock.

MATERIALS AND METHODS

Three isogenic strains of crossveinless-like (cvl) Drosophila melanogaster; "Orinda X, Orinda II, Orinda III"; "Orinda X, Orinda II, cvl-5-hi III"; and "cvl-5-hi X, cvl-5-hi II, cvl-5-hi III (Curly float)"; hereafter referred to as Ona X, Ona II, Ona III; Ona X, Ona II, 5-hi III; and 5-hi X, 5-hi II, 5-hi III respectively; were used in the experiments to be described. The cvl phenotypes of Ona X, Ona II, 5-hi III and 5-hi X, 5-hi II, 5-hi III depend upon a major gene on chromosome III and polygenic modifiers on the X and chromosome II (Mohler 1965b). These strains were constructed by Mohler according to the procedure outlined by him, involving the use of balancer chromosomes (1965b). For the Ona X, Ona II, Ona III, all three chromosomes were taken from the Orinda wild-type. For the Ona X, Ona II, 5-hi III, the X and chromosome II were taken from the wild-type, and the third chromosome was taken from the 5-hi selection line, thus the major gene is present in this strain. For the 5-hi X, 5-hi II, 5-hi III strain, all three chromosomes were taken from the 5-hi selection line. The 5-hi X, 5-hi II, 5-hi III stock also contains the Curly (Cy) chromosome, a multiply inverted second chromosome, which was added to enhance fertility in this stock.

Cultures of these strains were maintained in stock bottles on the standard cornmeal - agar - molasses - propionic acid medium.

Experimental cultures were handled in the following manner: mass matings (approximately half females and half males) were placed in culture bottles and subcultured every three to four days. After about three consecutive subculturings new flies, from stock sources, were used in order to maintain a high level of fertility and to produce adequate numbers of pupae, and also to insure against contamination. The eggs and larvae developed at 25°C. in constant temperature incubators on the standard medium supplemented with a thick suspension of yeast on the fourth or fifth day of incubation.

White prepupae were collected and placed in shell vials (25 x 95 mm) and aged in a Precision Scientific constant temperature water bath at 23.0°C. ± 0.1°C. Whiteness of the prepupae indicates that it was collected within one hour after the onset of puparium formation, and the time of collection is taken as time zero in pupal development. Temperature shocks were carried out in constant temperature water baths, either in shell vials or teabags; warmup time in vials is approximately two minutes and in teabags two seconds or less (Milkman, 1963). After the pupae were subjected to temperature shock they were replaced in the 23° water bath and remained there for 24 hours, after which time they were allowed to continue development at 25°C. in a constant temperature incubator until eclosion.

After eclosion the adult flies were rated as to the extent of

missing crossvein (expressivity), the percentage of flies showing interruption (penetrance), and the site of interruption (specificity). The rating is carried out by estimating the fraction of crossvein missing, by arbitrarily dividing the posterior crossvein into fifths, so that a rating of zero indicates the entire crossvein is present in both wings, while a rating of ten indicates total lack of posterior crossvein in both wings. The following symbols are used to represent the various statistics: n=number of flies; %=penetrance; $\bar{r}_{10}$=mean rating among all flies; and r'_{10}=mean rating among flies showing interruptions.

The following specific types of experiments will be described in further detail: age response, dose response, split treatments, and localization of the major gene on chromosome III.

Age Response

Pupae were collected over a range of from 14 hours through 34 hours of pupal development. At each collection two samples of 40 were obtained for treatment and one sample of 30 for control. At the appropriate developmental age the pupae were subjected to temperature shock for 20 minutes at 40.5°C. In this manner peak periods of sensitivity to heat shock could be obtained.

Dose Response

Pupae of one developmental age were collected in the manner described above, and were subjected to various durations of a given temperature. Temperatures used here, were the following: 43.5°, 42.5°, 41.5°, 40.5°, 39.5°, and 38.5°C. For very short durations, treatments were carried out in teabags.

Split Treatments

Pupae collected in the manner described above were first subjected to temperature shock at one age in development, returned to the 23° water bath, and after a period of time were again subjected to temperature shock, after which they were returned to the 23° water bath and development continued as described previously.

Genetic Localization

Localization of the major gene on the third chromosome was done as follows: Ona X, Ona II, 5-hi III females were mated to ru h th st cu sr e^s ca ("rucuca") males (see Table 1 for description of mutants). The F_1 females were mated to "rucuca" males in order to obtain crossovers and also to check if the cvl-5-hi major gene might be associated with a structural chromosome change. The single crossovers and some appropriate multiples were then

mated, as males, back to Ona X, Ona II, 5-hi III females to determine the presence or absence of crossveinlessness and to localize the major gene to a specific point.

Table 1. A listing of the mutant alleles and their phenotypes used in the localization of the cvl-5-hi-III major gene (Bridges and Brehme, 1944, p. 248-250).

Genetic symbol	Name of mutant	Locus	Phenotype
ru	roughoid	0.0	Eyes small; rough; errupted facets.
h	hairy	26.5	Extra hairs, on scutellars, veins, pleurae, head.
th	thread	43.2	Aristae threadlike, without branches.
st	scarlet	44.0	Eye color scarlet, ocelli white.
cu	curled	50.0	Wings upcurved, body dark, posterior scutellars crossed.
sr	stripe	62.0	Dark dorsal stripe.
e^s	ebony-sooty	70.7	Body color black.
ca	claret	100.7	Eye color clear ruby.

RESULTS

The results are presented in four parts; first, identification of the responses to temperature shock in age response studies and the specificity of the responses; second, characterization of the separate responses; next, a preliminary study of the extent and identity of the rapid crossvein restoring response and finally, a preliminary genetic study of the responses. Except for the final section on the genetic studies, all experiments discussed were carried out with Ona X, Ona II, 5-hi III.

Type of Responses

As shown in Figure 1, this strain responded to 20 minute heat shocks, a subthreshold treatment in wild-type (Milkman, 1961), with three responses at different times in development: (1) Heat shocks at 14 hours in pupal development restored the wild-type phenotype. (2) Heat shocks at 22 hours produced a more extreme crossveinless phenotype. (3) Heat shocks from 28 hours through 34 hours in pupal development also restored (i.e., showed a reduction in mean rating). An examination of Table 2 shows that the difference between the control and treatments during this period of development is due to a reduction in penetrance (%) without any apparent change in expressivity (r'_{10}). In order to test this, two analyses were performed

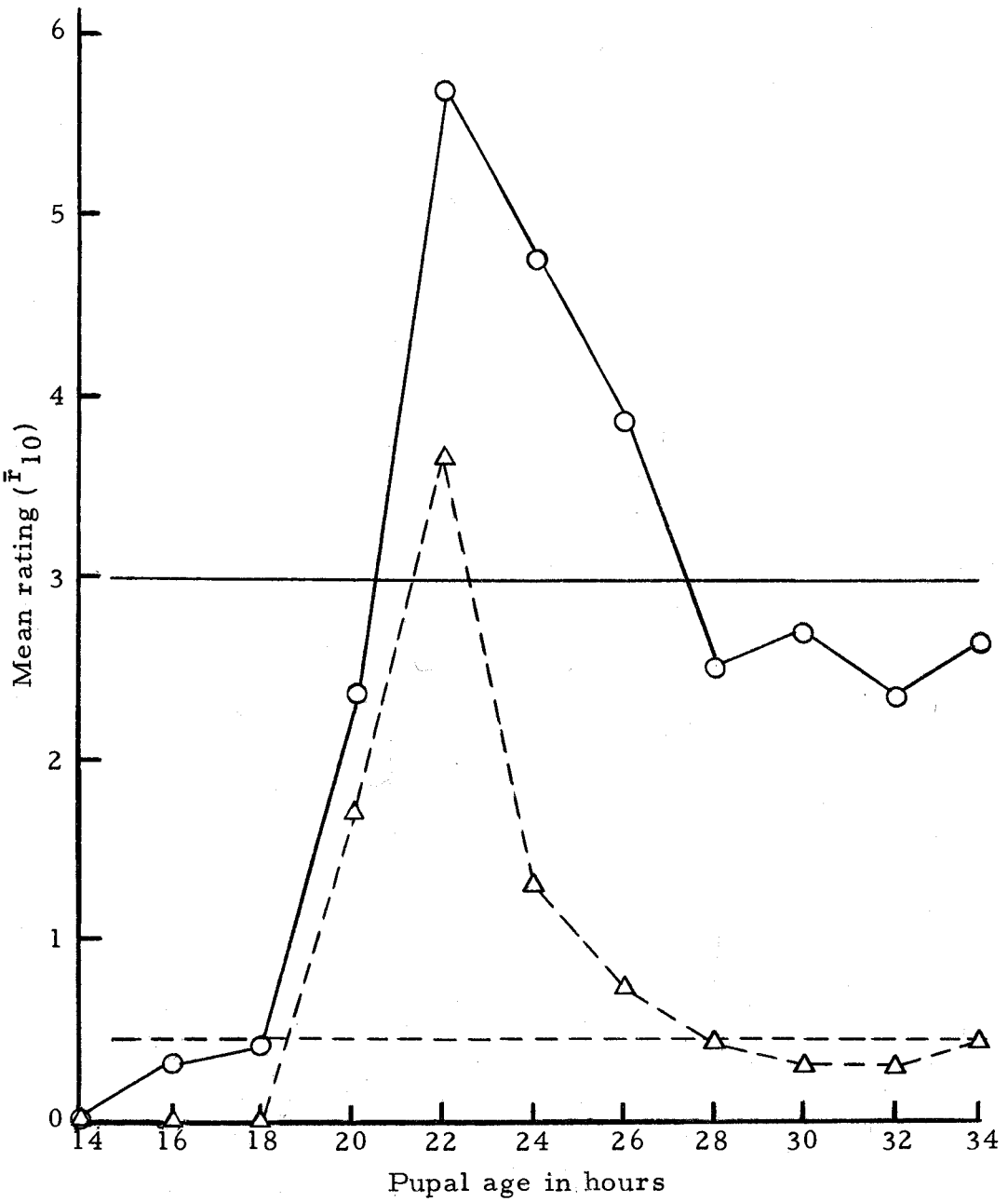

Figure 1. Age response of Ona X, Ona II, 5-hi III to 20 minute temperature shock (40.5°). Horizontal lines are the spontaneous control levels. Females, circles and solid lines; males, triangles and dotted lines.

over this range of pupal development. First, an analysis using the Mosteller-Tukey method of determining individual standard errors by means of binomial probability paper (1949), showed that among the samples treated at these ages there is no significant variation with age. Second, an analysis of variance showed that there was no difference in expressivity between the control and treatments among those flies showing interruptions.

Table 2. Age response of Ona X, II, 5-hi-III to 20 minute shocks over the range of pupal ages between 14 and 34 hours.

	Females			Males		
Age	n	%	r'_{10}	n	%	r'_{10}
14	49	2.4	2.0	22	0	0
16	141	16.3	1.8	84	0	0
18	139	26.6	1.5	123	0.8	2.0
20	140	52.9	4.5	105	37.1	4.6
22	130	89.2	6.4	116	81.9	5.2
24	144	82.6	5.7	103	30.1	4.4
26	140	77.1	5.1	99	19.2	3.6
28	187	62.6	4.0	124	13.7	3.1
30	192	74.5	3.6	141	13.5	2.4
32	202	65.4	3.5	150	9.3	2.3
34	150	75.3	3.5	122	7.4	2.2
Cont.	842	88.4	3.4	695	21.2	2.0

Because Mohler (1965a) observed differential responses at the two longitudinal veins following temperature shock in cvl-6b, such differences were looked for in this strain. The specificity of cvl phenotypes are shown in Table 3. The various responses are clearly expressed differently at the two ends of the crossvein, i.e., at the fifth longitudinal vein (L5) and at the fourth longitudinal vein (L4).

Table 3. Changes in specificity during age response of Ona X, Ona II, 5-hi III. 20 minute treatment with 40.5°. N = number of wings, % = percent of wings with particular type of interruption.

	Females			Males		
Age	N	%L4	%L5	N	%L4	%L5
14	98	1.0	0.0	44	0.0	0.0
16	282	0.0	0.8	168	0.0	0.0
18	278	0.7	1.0	246	0.4	2.8
20	280	25.4	27.5	210	17.6	18.6
22	260	68.8	58.9	232	50.9	47.0
24	288	57.3	44.1	206	14.6	17.5
26	280	48.9	24.6	198	9.1	6.1
28	374	35.3	16.6	248	6.1	3.6
30	384	43.0	15.1	282	4.6	2.8
32	404	41.3	8.9	300	2.0	3.3
34	300	51.7	7.7	244	0.8	3.7
Cont.	1682	20.6	68.3	1390	0.9	12.1

An interesting observation upon the pattern of sex differences can be made from Figure 1 and Table 3. In this pattern the responses of the sexes produced quantitatively similar phenotypes up through 22 hours, but after that age the responses, though qualitatively similar, produce phenotypes quantitatively as different as the control phenotypes.

The age response study has demonstrated three different responses to temperature shock at different times in development; a crossvein restoring response at 14 hours, a crossvein limiting response at 22 hours, and a reduction in penetrance at 28 through 34 hours in pupal development. Concurrent with these different responses are characteristic changes in specificity.

Characterization of the Separate Responses

Pupae treated with 40.5° at 22 hours show three responses dependent upon the duration of the treatment. (1) Short durations, from one minute up to five minutes cause restoration of the crossvein. (2) Durations of up to 20 minutes produce more extreme crossveinlessness. (3) Durations longer than 20 minutes have a crossvein restoring response. For the detailed information see Figure 2 and Appendix Table 3.

Changes in specificity following different durations of temperature shock at 22 hours of pupal development are shown in Table 4;

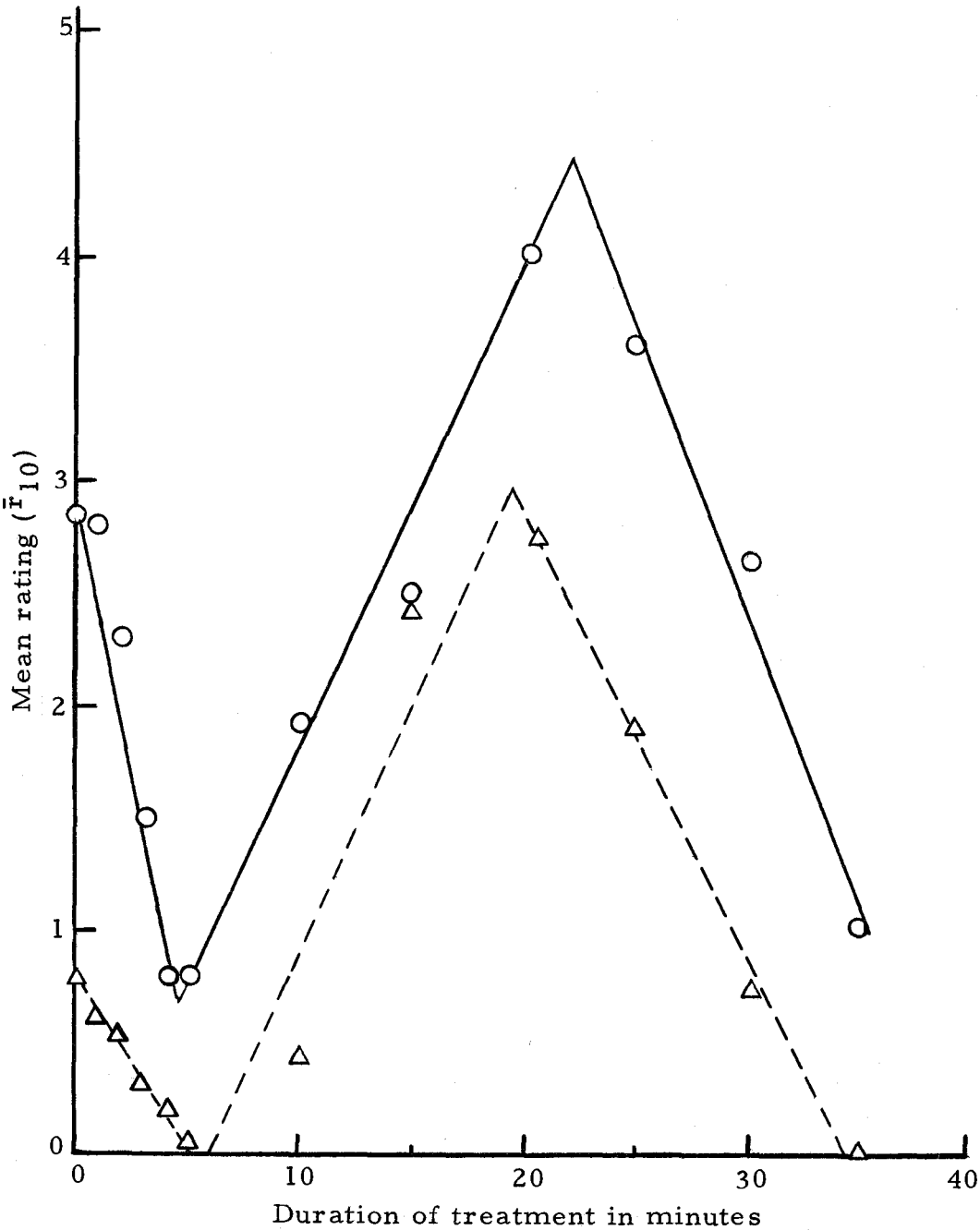

Figure 2. Dose response of Ona X, Ona II, 5-hi III at 22 hours of age, treatment 40.5°. Females, circles and solid lines; males, triangles and dotted lines.

indicating the various responses are expressed differently at the two longitudinal veins.

Table 4. Changes in specificity during dose response of Ona X, Ona II, 5-hi III. 22 hours of age, treated with 40.5°.

Duration	Females			Males		
minutes	N	%L4	%L5	N	%L4	%L5
1	90	27.8	68.9	54	0.0	20.4
2	70	25.7	55.7	66	1.5	16.7
3	78	12.8	42.3	68	1.5	8.8
4	82	7.3	24.4	62	1.6	6.5
5	92	9.8	15.2	56	1.8	0.0
10	66	31.8	16.7	58	5.2	6.9
15	60	33.3	23.3	46	34.8	19.6
20	72	50.0	37.5	64	35.9	25.0
25	62	46.8	30.6	42	26.2	19.1
30	40	25.0	25.0	42	11.9	4.8
35	8	12.5	12.5	6	0.0	0.0
40	1	0.0	0.0	0	---	---
Cont.	312	19.6	73.7	264	1.1	31.1

It is well known that denaturation of a protein occurs at high temperature with very high activation energies. Indeed, Milkman (1961, 1962, 1963) concluded from temperature dose studies that the phenocopy induction in wild-type Drosophila was due to a series

of changes in the tertiary structure of a protein. In order to test whether the various responses demonstrated in this study are also due to conformational changes in a protein (or proteins) temperature coefficients for the responses were determined.

Temperatures at one degree intervals between 38.5 and 43.5° (six in all) were compared as to their effect on the crossvein limiting response and the crossvein restoring responses, and it was found that all temperatures at 22 hours in development gave qualitatively the same result as that depicted in Figure 2, see Appendix Tables 1, 2, 3, 4, 5, and 6.

First, a temperature coefficient for the crossvein limiting response at 22 hours of pupal development was calculated by determining the least squares estimates of the regression lines for 38.5 and 42.5° treatments, and measuring the ratio between treatment durations giving identical rating values. This calculation gave a temperature coefficient of 1.9 per degree rise in temperature (Q_1), which corresponds to an activation energy of about 126,000 calories per mole (calculation based on the Arrhenius equation, cited in West, 1963, p. 413). These calculations are characteristic of conformational change of a protein due to heat denaturation (Eyring and Stearn, 1939), and are therefore interpreted as such. By converting the treatments of the various temperatures to equivalent times at 40.5°, the data are plotted with response as a linear function of time as

shown in Figures 3 and 4. The fit tests the validity of the temperature coefficient of 1.9. Because they overlap, the male and female plots are given separately.

Second, by comparing time to equivalent responses between the various temperatures, it was found that a temperature coefficient (Q_1) of 2.1 fits the data quite well for the rapid crossvein restoring response at 22 hours. Converting the treatments of the various temperatures to equivalent times at 40.5°, all the data can be plotted with response as a linear function of time, see Figure 5, again showing the reliability of the calculated temperature coefficient. A Q_1 of 2.1 corresponds to an activation energy of about 146,000 calories per mole which is consistent with conformational change of a protein due to thermal denaturation.

Third, a temperature coefficient (Q_1) of at least 1.7 was calculated for the crossvein restoring response following long durations of temperature shock at 22 hours (calculations based on Appendix Tables 1, 2, 3, 4, 5, and 6). However, this value of 1.7 is reliable only as a minimal estimate because the calculation is confounded by the fact that very long durations of heat shock cause death, and by the fact that at the lower temperatures (38.5 and 39.5°) the onset of this response is retarded, while at the higher temperatures (42.5 and 43.5°) the responses occur so rapidly as to be almost unmeasurable. Nevertheless, a temperature coefficient of 1.7 would indicate

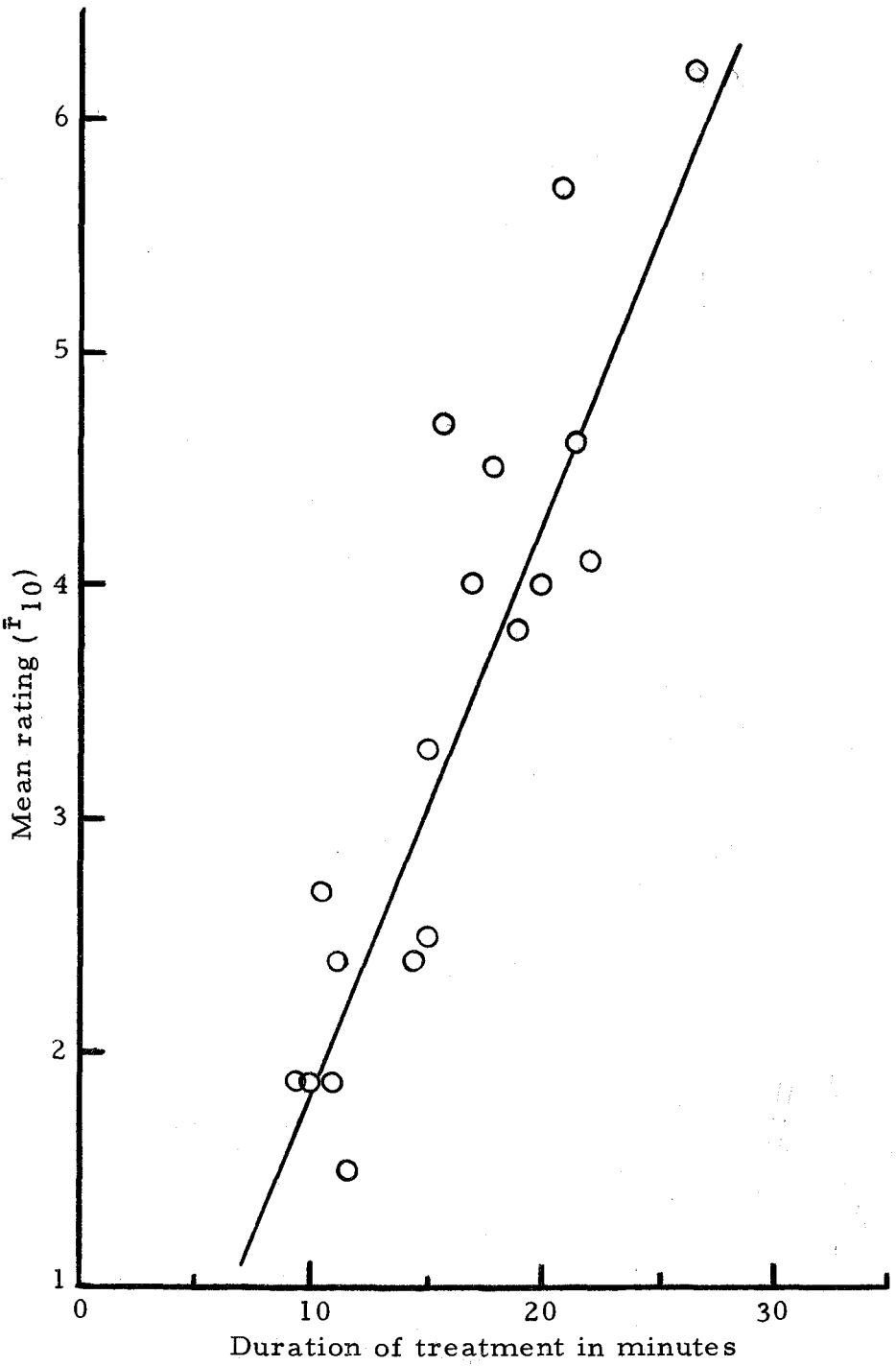

Figure 3. Dose response of Ona X, Ona II, 5-hi III females at 22 hours of age. Crossvein limiting response, all temperature data converted to equivalent times at 40.5°. $Q_{\bar{r}}$ = 1.9.

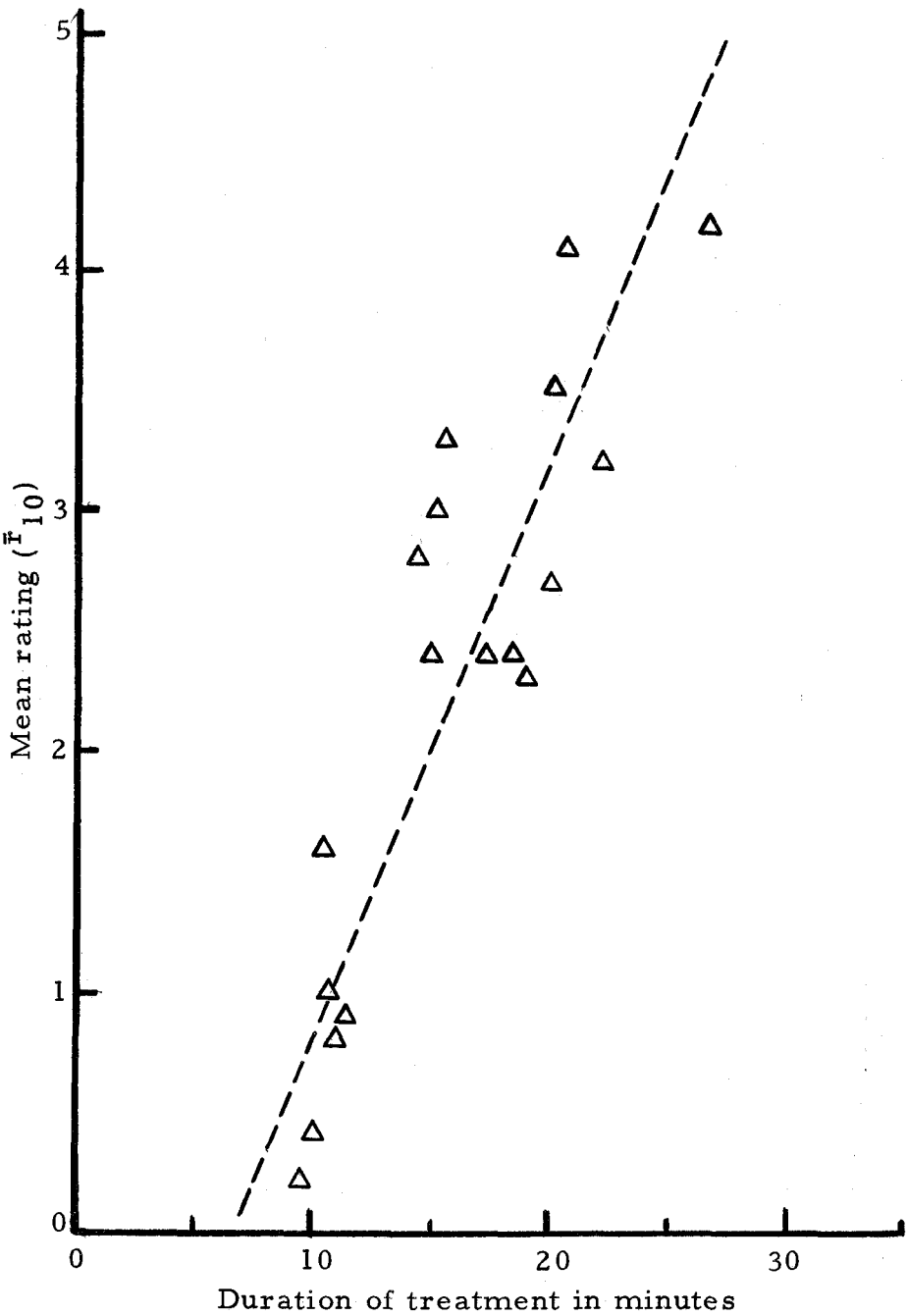

Figure 4. Dose response of Ona X, Ona II, 5-hi III males at 22 hours of age. Crossvein limiting response, all temperature data converted to equivalent times at 40.5°. Q_1= 1.9.

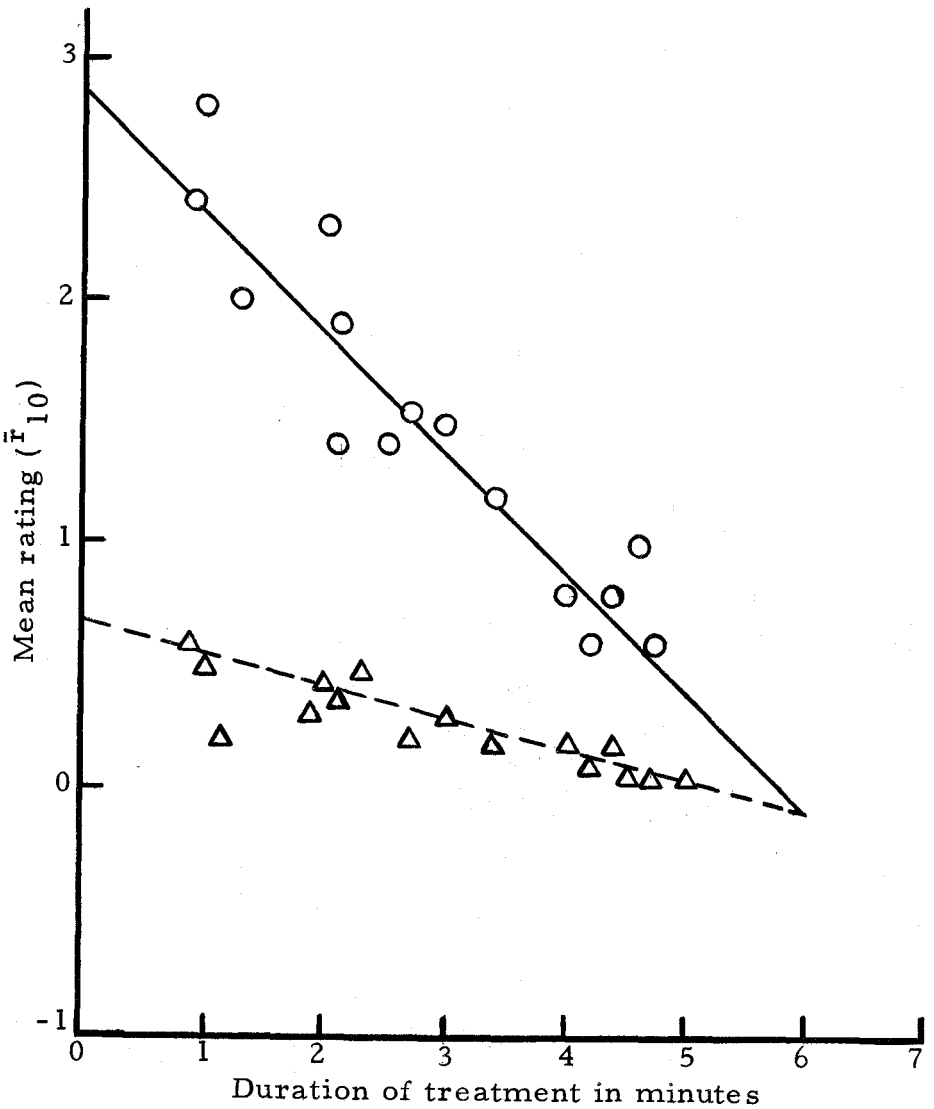

Figure 5. Dose response of Ona X, Ona II, 5-hi III at 22 hours of age. Rapid crossvein restoring response, all temperature data converted to equivalent times at 40.5°. $Q_1 = 2.1$. Females, circles and solid lines; males, triangles and dotted lines.

conformational changes in a protein as the basis of the second restoring response.

Dose response studies at 22 hours of pupal development have demonstrated three responses depending upon the duration of treatment, a rapid crossvein restoring response, a crossvein limiting response with intermediate durations, and a crossvein restoring response following long durations of temperature shock. All of these responses have characteristic changes in specificity, and they are consistent with the interpretation of conformational changes in a protein due to thermal denaturation.

Temperature shocks at 14 hours of pupal development show only one response, rapid crossvein restoration, see Figure 6 and Appendix Tables 7, 8 and 9. The temperature coefficient calculated for this response is identical with that of the rapid restoration response at 22 hours, $Q_1 = 2.1$, and the data also fit the same lines as the rapid restoration response of 22 hours shown in Figure 5. Thus, it appears that what is being affected at 14 hours in development is also affected at 22 hours. The recognition that these two responses to short term temperature shock are probably identical leads to the question as to the extent of the rapid restoration response in pupal development. This will be explored in more detail later.

Temperature shocks at 28 hours in pupal development show

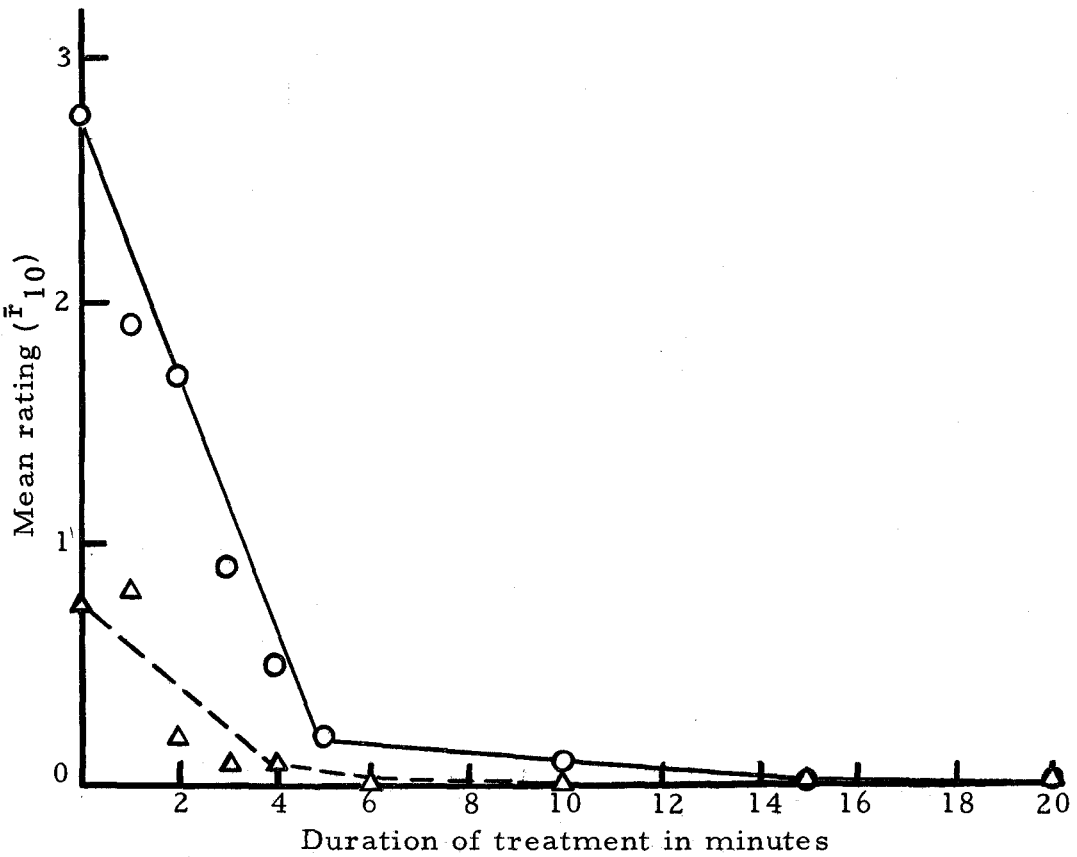

Figure 6. Dose response of Ona X, Ona II, 5-hi III at 14 hours of age, treatment 40.5°. Females, circles and solid lines; males, triangles and dotted lines.

two responses; first, an expected reduction in penetrance with durations up to 20 minutes just as demonstrated in Figure 1 with the age response study and, second, an unexpected crossvein limiting response with durations beyond 20 minutes as can be seen in Figure 7 and Table 5. This response which requires a certain threshold of temperature shock (20 minutes) is actually similar to that found at 25 hours with Oregon-R (see Milkman, 1962). No temperature study has been performed with the responses at 28 hours, so that it is not yet possible to identify them with changes in conformation of a protein.

Table 5. Dose response of Ona X, Ona II, 5-hi III. 28 hours of age treated with 40.5°.

Duration	Females			Males		
minutes	n	%	r'_{10}	n	%	r'_{10}
5	31	74.2	2.9	38	23.7	2.2
10	40	72.5	2.7	33	9.1	1.0
15	40	50.0	2.8	31	19.4	1.7
20	32	34.4	3.3	37	13.5	1.4
25	38	60.5	3.7	30	13.3	3.3
30	40	92.5	4.2	27	74.1	3.5
35	38	97.4	5.2	14	85.7	3.9
40	18	100.0	7.4	15	100.0	6.5
Cont.	69	76.8	2.6	58	17.2	1.6

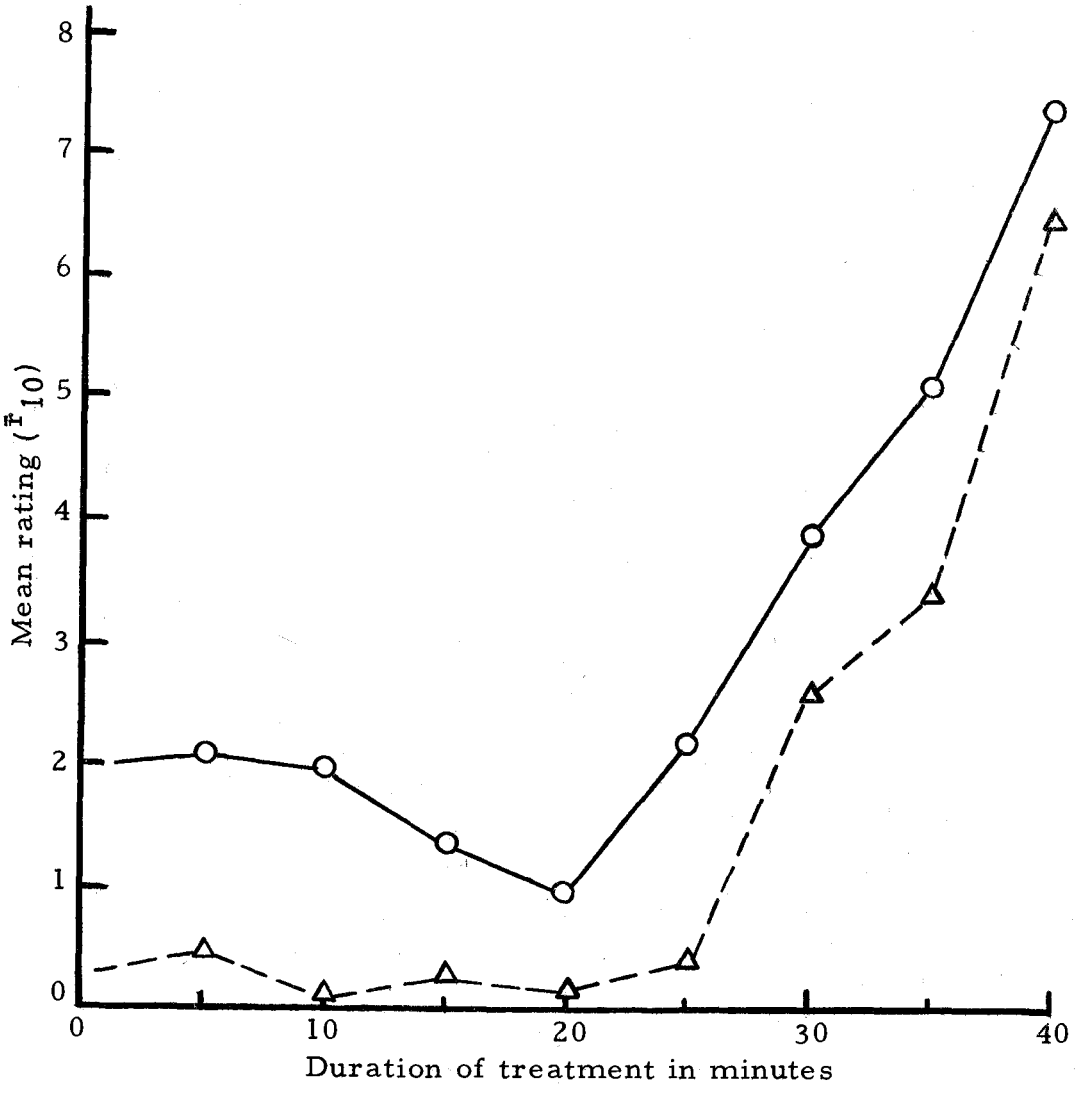

Figure 7. Dose response of Ona X, Ona II, 5-hi III at 18 hours of age, treatment 40.5°. Females, circles and solid lines; males, triangles and dotted lines.

Extent and Identity of the Rapid Restoration

With the recognition that the rapid crossvein restoring responses at 14 and 22 hours are likely identical the question arose as to the extent and identity of the rapid crossvein restoring responses.

Pupae subjected to five minute heat shocks (40.5°) responded by restoring crossvein over most of the range of pupal ages between six and 30 hours of development as seen in Figure 8 and Table 6. Thus, rapid restoration is characteristic of most of the period studied.

Table 6. Age response of Ona X, Ona II, 5-hi III. 5 minute shocks with 40.5°, over the range of pupal ages between 6 and 30 hours.

	Females			Males		
Age	n	%	r'_{10}	n	%	r'_{10}
6	27	63.0	2.6	13	61.5	1.9
8	42	35.7	2.0	32	15.6	2.4
10	25	28.0	3.1	17	5.9	2.0
12	28	25.0	2.4	17	0.0	0.0
14	26	3.9	1.0	19	5.3	1.0
16	26	15.4	1.8	26	0.0	0.0
18	37	24.3	1.6	22	0.0	0.0
20	30	26.7	1.6	22	0.0	0.0
22	31	25.8	1.8	27	3.7	1.0
24	28	28.6	2.0	27	0.0	0.0
26	29	41.4	1.4	28	3.6	1.0
28	18	55.6	3.0	38	7.9	1.7
30	28	75.0	3.0	27	14.8	1.8
Cont.	141	80.1	2.6	118	17.8	1.3

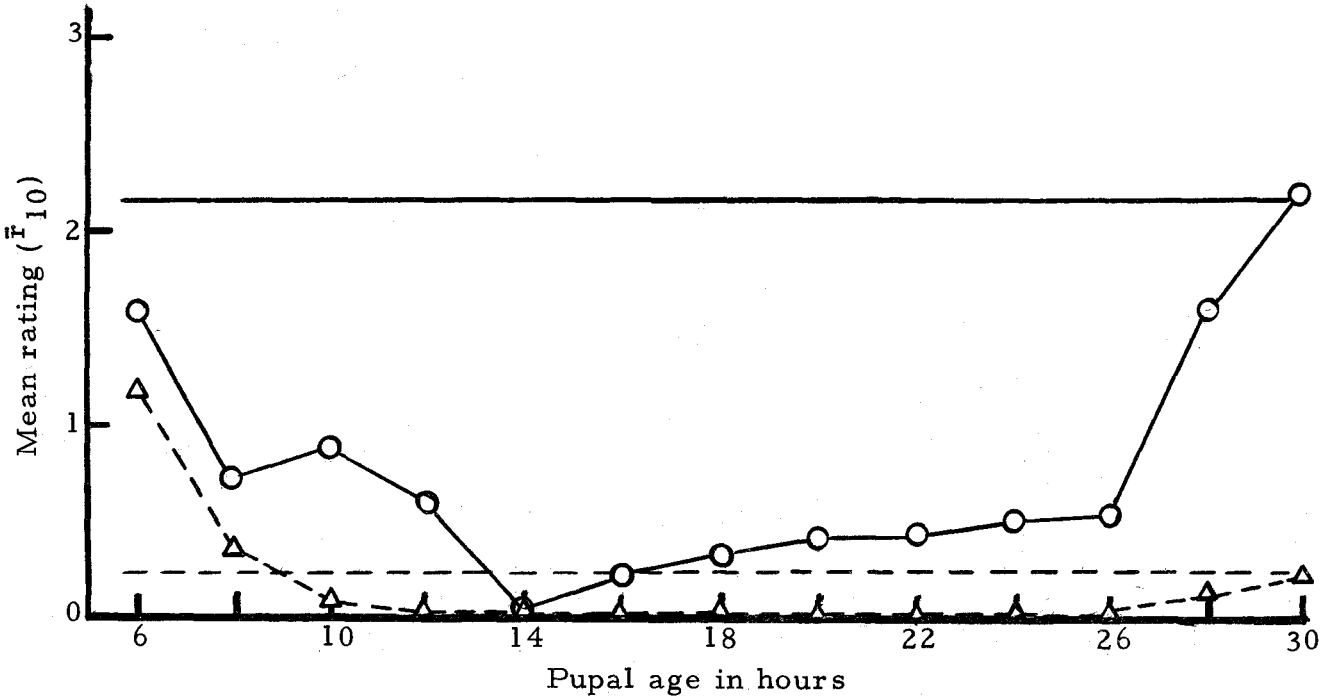

Figure 8. Age response of Ona X, Ona II, 5-hi III to five minute temperature shock (40.5°). Horizontal lines are the spontaneous control levels. Females, circles and solid lines; males, triangles and dotted lines.

Following the principles outlined by Milkman (1962, 1963), the apparent identity of the rapid response at 14 and 22 hours was tested by the use of split treatments, for if they are indeed identical they should both respond alike with additiveness.

Pupae subjected to five minute pretreatments (40.5°) at 14 hours of pupal development followed by treatments (40.5°) at 22 1/2 hours showed no increase in crossvein defects over the level observed at the 14 hour temperature shocks (see Table 7). Milkman (1962, 1963) reported like responses in wild type to split treatments and termed the phenomenon 'protection'. Pupae subjected to five minute pretreatments (40.5°) at 22 hours of pupal development followed by

Table 7. Effect of pretreatment on the response to temperature shock at 22 1/2 hours. Pre-treatment 5 minutes (40.5°) at 14 hours of age, treatment 40.5°.

Duration of treatment	Females				Males			
	n	%	$\bar{r}_{10}$	r'_{10}	n	%	$\bar{r}_{10}$	r'_{10}
10	36	25.0	0.4	1.6	28	0.0	0.0	0.0
15	36	25.0	0.6	2.4	19	0.0	0.0	0.0
20	42	28.6	0.7	2.4	30	0.0	0.0	0.0
25	41	19.5	0.5	2.0	33	3.0	0.1	3.0
30	47	19.2	0.6	3.3	31	0.0	0.0	0.0
35	36	16.7	0.4	2.7	19	0.0	0.0	0.0
Cont.	88	93.2	2.8	3.0	65	43.1	0.8	1.9

treatments (40.5°) at 22 1/2 hours showed an increase in crossvein defects, and the two treatments appear to be additive or super-additive in this case; see Figure 9 and Table 8 and compare with Figure 1. Further, in both of these experiments pretreatment protects against death, for the combined treatment of 40 minutes (5 minutes pretreatment + 35 minutes treatment) is lethal at 22 hours in a continuous treatment. Also, it is evident that pretreatment with five minutes of 40.5° produces like responses in the males and females, and they are more alike than in the control samples (see Figure 9 and Table 8).

Table 8. Effect of pretreatment on the response to temperature shock at 22 1/2 hours. Pretreatment 5 minutes (40.5°) at 22 hours of age, treatment 40.5°.

Duration of treatment	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
10	40	80.0	5.0	31	64.5	5.4
15	45	86.7	5.2	26	76.5	4.7
20	39	92.3	5.9	34	91.2	5.3
25	34	94.1	6.4	31	96.8	5.6
30	42	97.6	6.3	27	88.9	5.1
35	40	92.5	5.4	29	79.3	4.4
40	36	61.1	5.6	18	38.9	3.1
50	18	50.0	5.4	18	5.6	2.0
60	16	0.0	0.0	19	0.0	0.0
Cont.	68	89.7	3.2	77	40.3	1.6

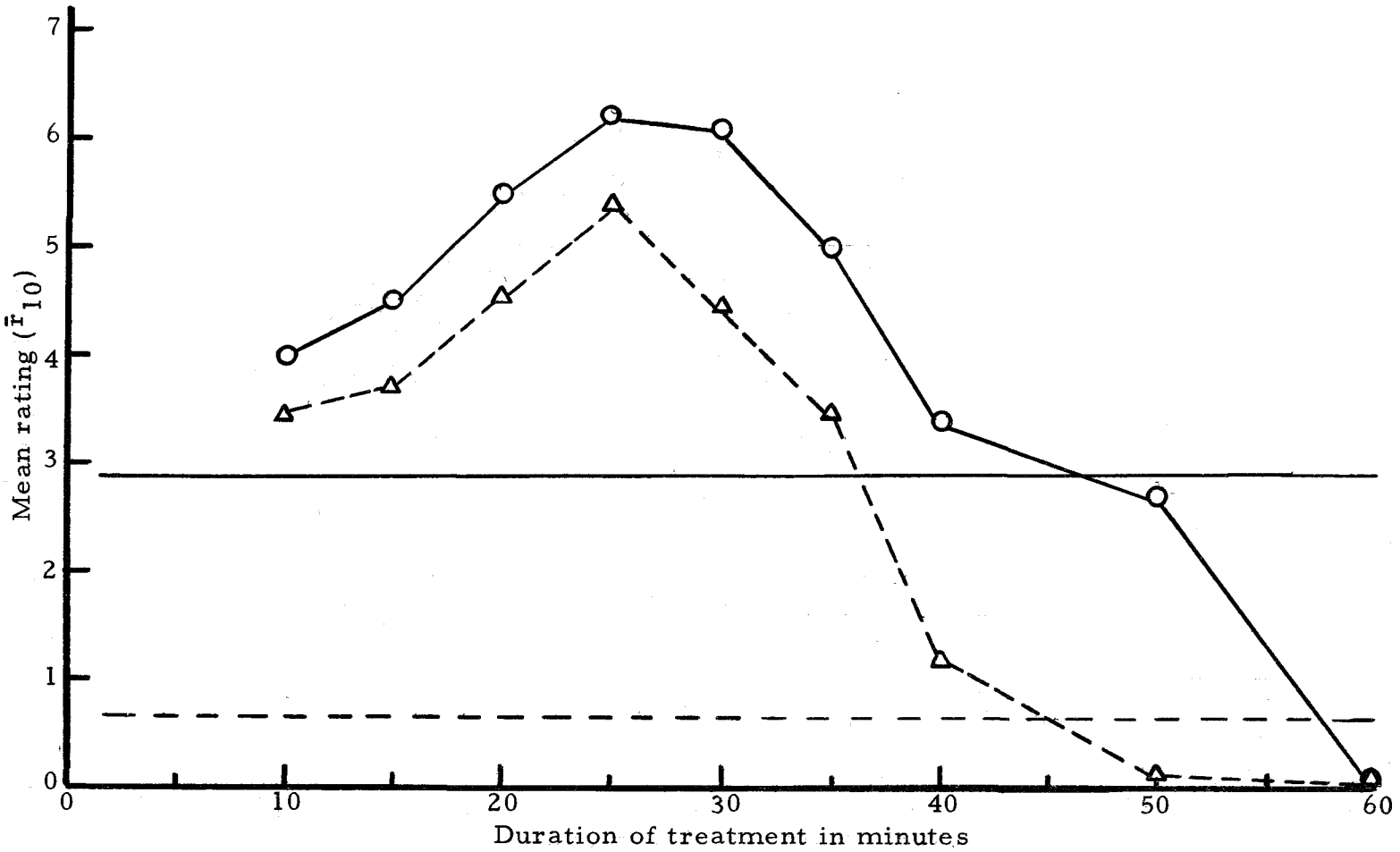

Figure 9. Split treatments with 40.5° on Ona X, Ona II, 5-hi III. Pretreatment five minutes at 22 hours, treatment at 22 1/2 hours. Horizontal lines are the spontaneous control levels. Females, circles and solid lines; males, triangles and dotted lines.

Although the results of the two split treatment experiments are different, it cannot be unequivocally stated that the two rapid crossvein restoring responses at 14 and 22 hours are different on the basis of these experiments, because Milkman (1963) has shown that the interval between treatments is important in determining whether additivity or 'protection' occurs with split treatments. However, both experiments should have given the same result for according to Milkman (1963) a 25 minute interval should have been long enough to determine protection. Obviously, further experiments are needed with split treatments to clarify this point.

The second of these two experiments with split treatments is interesting in that an increase of crossvein defects has been produced yet the flies were protected from death, Milkman (1963) was not able to produce such a response and concluded that the protein being affected is also involved in some vital process. Further, this method of approach, i.e., split treatments, may be the means of studying the crossvein restoring response following long durations of treatment at 22 hours, for the problem of death is eliminated in these experiments.

Preliminary Genetic Study of the Responses

The 5-hi X, 5-hi II, 5-hi III stock, which contains modifiers for increased penetrance and expressivity on the X and second

chromosome from the cvl-5-hi line, shows the same responses to 20 minute temperature shocks; see Figure 10, as already described in the age response study with Ona X, Ona II, 5-hi III. The changes in specificity following temperature shock are also like those observed with Ona X, Ona II, 5-hi III. This strain differs from the Ona X, Ona II, 5-hi III strain in having a greater expression and in that the peak period for the crossvein limiting response occurs at about 20 hours in pupal development, approximately two hours earlier than in Ona X, Ona II, 5-hi III. A similar difference of two hours between these two strains was observed by Mohler and Swedberg (1964) in stages of wing vein development, and by Wong (1966b) in temperature effective period studies.

Substitution of a wild allele for the major gene was accomplished by making a heterozygote with the cross of Ona X, Ona II, Ona III with Ona X, Ona II, 5-hi III. Pupae heterozygous for the major gene, showed no response to 20 minute (40.5^{o}) heat shocks. Thus it appears that a gene or genes on the third chromosome in the cvl-5-hi line have profound effects upon the response to temperature shock. Presumably the relevant gene is the major gene of the spontaneous effect.

As a first step toward the study of this point, experiments were undertaken to localize the major gene. The data from the "rucuca" test cross shows that the cvl-5-hi major gene is not

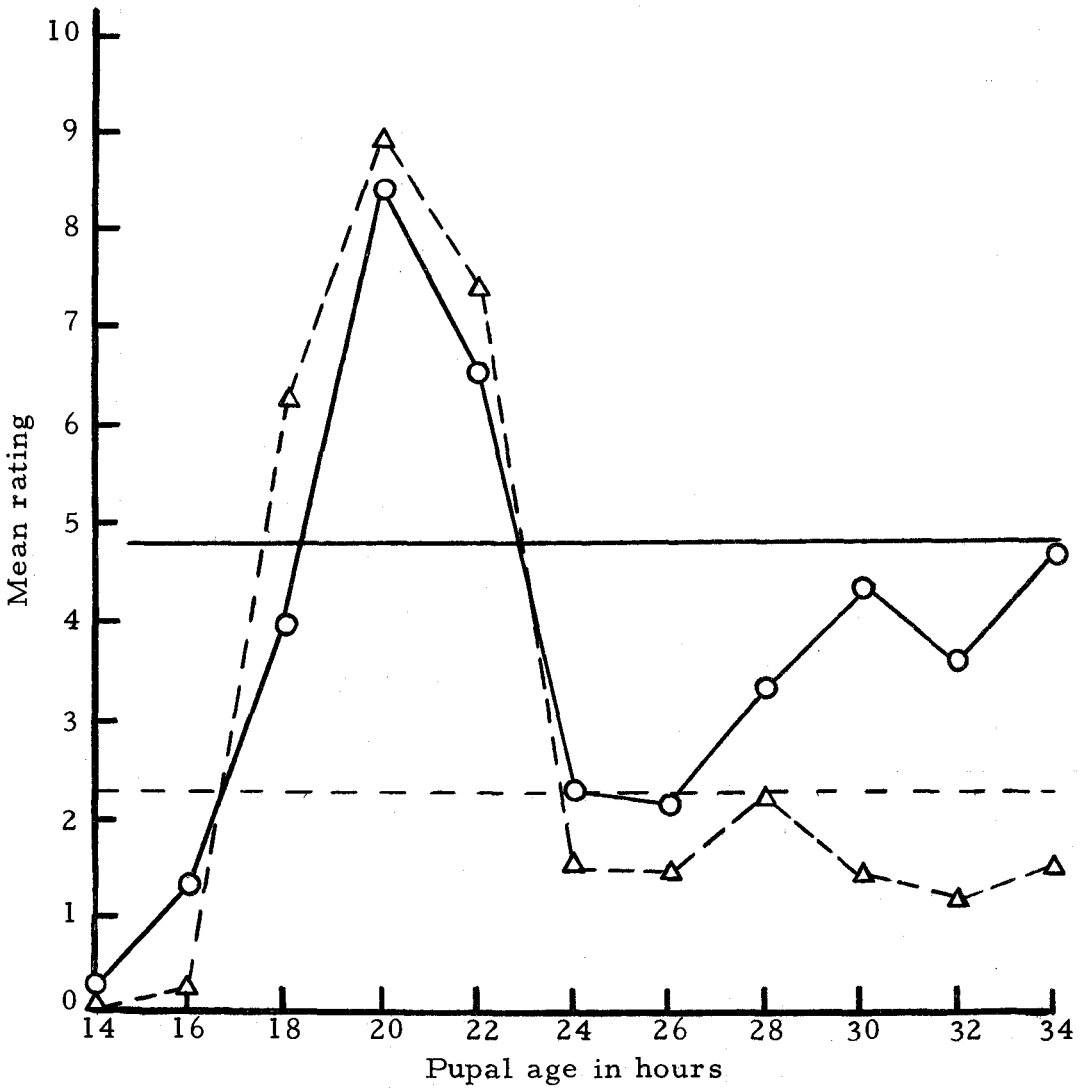

Figure 10. Age response of 5-hi X, 5-hi II, 5-hi III to 20 minute temperature shock (40.5°). Horizontal lines are the spontaneous control levels. Females, circles and solid lines; males, triangles and dotted lines.

associated with a structural chromosome change, as can be seen from a comparison of crossover data from the cross with Ona X, Ona II, 5-hi III and the cross with Ona X, Ona II, Ona III (see Table 9). Though region I crossovers between these two crosses gave different map distances, the difference is most likely due to classification error of roughoid (ru), rather than actual differences in the two chromosomes. Classification of roughoid depends upon the recognition of slightly smaller eye size, a rough appearance to the eye and interrupted eye facets. Classification was uncertain in the first experiment, but with experience classification was more sharp in the second experiment.

Table 9. Comparison of crossover data from Ona X, Ona II, 5-hi III and Ona X, Ona II, Ona III.

Region	I ru - h	II h - th	III th - st	IV st - cu	V cu - sr	VI sr - e^s	VII e^s - ca
Data from Ona X, Ona II, 5-hi III	184	223	12	49	134	99*	331
Total = 1060							
Calculated map dist.	17.4	21.0	1.1	4.6	12.6	20.8	31.2
Data from Ona X, Ona II, Ona III	261	215	12	68	131	114**	374
Total = 1102							
Calculated map dist.	23.7	19.5	1.1	6.2	11.9	23.7	33.9

* Total = 476
** Total = 482

First, the backcross data from the single crossovers of the Ona X, Ona II, 5-hi III cross with Ona X, Ona II, 5-hi III shows that the cvl-5-hi major gene lies between st (44.0) and cu (50.0). Important in this conclusion was the fact that cu sr e^s ca recombinants showed some flies which carried the cvl-5-hi major gene and some which did not, indicating the gene must be somewhere between st and cu (for the detailed information see Table 10). Next, recombinant chromosomes with a crossover between st and cu were selected

Table 10. Preliminary genetic localization of the cvl-5-hi major gene.

Tested recombinant	Carried Cvl	Carried Cvl^+
ru h th st cu sr e^s ca	-	+
+ + + + + + + +	+	-
ru + + + + + + +	+	-
ru h + + + + + +	+	-
ru h th st + + + +	+	-
ru h th st cu + + +	-	+
ru h th st cu sr e^s +	-	+
+ + th st cu sr e^s ca	-	+
+ + + + cu sr e^s ca	+	+
+ + + + + sr e^s ca	+	-
+ + + + + + e^s ca	+	-
+ + + + + + + ca	+	-

+ indicates recombinant carried this allele of cvl-5-hi.
- indicates recombinant did not carry this allele of cvl-5-hi.

to refine the localization (see Table 11). Sixty-nine percent (38/55) of these crossovers occurred between st and the cvl-5-hi major gene and 31 percent (17/55) of the crossovers occurred between the cvl-5-hi major gene and cu. Sixty-nine percent of the map distance between st and cu (6.0 map units) is approximately 4.1 map units. Thus cvl-5-hi lies 4.1 map units to the right of st at 48.1 (i.e., 44.0 + 4.1) on chromosome III.

Table 11. Test cross data involving crossovers between st and cu.

Tested recombinants	Number with cvl	Number with cvl^+
+ + + + cu + + +	1	3
+ + + + cu + e^s +	0	1
+ + + + cu + + ca	1	2
+ + + + cu sr e^s +	2	3
+ + + + cu sr e^s ca	4	6
ru + + + cu sr e^s +	1	0
ru + + + cu sr e^s ca	0	2
ru h + + cu sr e^s ca	0	3
ru h th st + + e^s +	2	0
ru h th st + + + ca	3	2
ru h th st + + e^s ca	3	2
ru h th st + + + +	9	0
ru + th st + + + +	1	2
+ h th st + + + +	0	1
+ + + st + + + ca	0	1

DISCUSSION

There is a good deal of evidence supporting the conclusion that every cell of a multicellular organism normally carries the full complement of genetic information, yet the cells of higher organisms become very different in structure and function during the development of the mature organism. Thus, it is imagined that differential activity of the genes must take place.

Considerable information has been presented in the last decade showing that the "puffing" patterns of the polytene chromosomes of dipteran salivary glands reflect the patterns of differential gene activity in this tissue (Beermann, 1961). These "puffing" patterns can be specifically altered by compounds such as ecdysone, the molting hormone of insects, and it has been suggested that the acitivity of these genes may be regulated by such hormonal action (Karlson, 1965). Further, it has been shown that stress such as temperature shock and ionic solutions outside the normal physiological range can also change the "puffing" patterns (Ritossa, 1962; Berendes, et al., 1965).

Hadorn (1965) has shown with transplants of larval imaginal discs in Drosophila that differential gene activity is not permanent, for the primary determination of these anlage can be changed after repeated subculture.

It is evident that differentiation requires some means of defining when and what is formed in development. Paigen and Ganschow (1965) have listed four different kinds of genes which could be involved in this process: (1) Structural genes, which determine the kind of protein formed. (2) Regulatory genes, which would specify the nature of the control system to which the protein or its synthetic apparatus responds as part of its normal function in the cell. (3) Architectural genes, which would be concerned with the agents responsible for the integration of a protein into the structure of cells. (4) Temporal genes, which would control the time and place at which the three primary gene classes are activated, and would provide the program for the sequential events in differentiation.

Another facet of gene control or regulation is recognized at the morphological level by a constancy of phenotype despite the existence of considerable genetic and environmental variation. Waddington (1961) has termed such phenotypic constancy "canalization". The existence of a canalized phenotype involves the concept of a threshold, for if there were not areas where the phenotype was recognizable outside the area of constancy, analysis of the phenomenon would not be possible. Rendel (1959, 1962) has developed the idea of "make", and has defined it as that which develops or tends to develop a phenotype, and it is assumed that "make" is distributed

in a normal fashion under the area of canalization. Thus a threshold can be defined as that point where change in phenotype with a change in "make" is no longer linear. Outside the canalized region, the change in phenotype is linear with an increase in "make", while inside the canalized region there is no change in phenotype accompanying change in "make". Selection of genes affecting a phenotype is interpreted as shifting "make" along a linear scale, and this implies a limited amount of product, "make", is produced according to the requirements set by the genotype.

A threshold is also recognized in the crossveinless system (Milkman, 1960a), however here the hypothetical material "crossvein - making ability", though distributed in a normal fashion, was assumed to be produced in excess but only a certain amount is usable. Thus selection for genes affecting this character, or temperature shock destroying this character, changes the amount of usable material, and various levels of phenotypic change outside the canalized area are recognized.

Within the concept of a canalized phenotype are recognized a number of types of gene interaction. Interaction between allelic genes is called dominance and this may act as a consequence of control, for from classical genetics it has been shown that heterozygotes for a recessive gene have a phenotype which is like that of the homozygous dominant, i. e., AA = Aa $\neq$ aa. Mohler (1966) is

showing in cvl strains that the major genes are recessive to their wild alleles and in the two strains, cvl-6 and cvl-5, the double homozygote for the major genes are more extreme in expression. Further, he is demonstrating that in all modifier backgrounds +/cvl-6, cvl-5/cvl-5 is like +/+, cvl-5/cvl-5 and cvl-6/cvl-6, +/cvl-5 is like cvl-6/cvl-6, +/+ in level of expression, so that he has concluded that the addition of a heterozygous major gene does not shift the amount of "make", and the major genes appear to be regulated at a level of control antecedent to the control imposed by the thresholds of the crossvein.

Another type of interaction occuring within a canalized phenotype is epistasis, a term used to describe non allelic gene interaction. A classic example of epistasis is demonstrated in coat color of mammals, if the animal is homozygous for the recessive allele for albinism further gene substitution for coat color variation is not recognized. It is conceivable that epistasis might act in a similar fashion in the canalized phenotype.

Because biochemistry is basic, the mechanisms whereby differential gene activity is controlled will be better understood at this level, and a survey of the models of gene regulation will next be discussed from this point of view.

Support for the supposition that there are specific genetic elements which control differential gene activity has been presented

by Jacob and Monod (1961) in the control of β-galactosidase synthesis in Escherichia coli. The model as proposed by these investigators postulates the presence of a regulator gene which produces a repressor molecule (presumably protein in nature) which can exist in two states, one being inactive in the repression of the biosynthesis of the enzyme, the other being active in repression. Supposedly the repressor molecule can act in either of two ways; (1) by inhibiting the synthesis of mRNA at yet another gene (or gene region), the operator, whose function is to start the synthesis of mRNA at the structural genes determining the production of the enzyme; or (2) by combining with the mRNA of the structural genes and thus inhibiting synthesis of the enzyme. However, if a small molecule, an inducer is present in the system it can combine with the repressor molecule, thus derepressing the system and allowing the operator gene to start the synthesis of mRNA at the structural genes.

An alternative method of control which has been demonstrated in microorganisms is the phenomenon known as "feedback inhibition", in which the end product regulates the reaction chain which gave rise to it.

Although these models stimulate speculation as to what is occurring in higher organisms there has of yet been no direct demonstration of regulator genes which function in the manner

described above. Though protein synthesis has been demonstrated to be regulated at the chromosomal level in higher organisms (at least DNA-dependent RNA synthesis) by nuclear histones (Huang and Bonner, 1962), there is an apparent lack of control of enzyme activity in mammalian systems, for, Paigen and Ganschow (1965) have pointed out that heterozygotes for an enzyme defect show an intermediate level of activity where one would expect the same level as in the homozygous normal. Bacteria, on the other hand, are regulated to produce a certain level of enzyme even in artificial diploid conditions (heterogenotes).

This may mean that in higher organisms we are not yet able to determine what phenomena are interpretable at the biochemical level, and it is still helpful to explore morphology in order to devise defining operations which may recognize control. Any interpretation of such morphological events in terms of the models as presented in microorganisms must, however, be made with caution.

The crossveinless system has been studied in some detail and is normally a canalized phenotype, but can be changed by selection of genes which disrupt the constancy of phenotype. Studies on the development of wing venation in wild-type and crossveinless-like strains (Mohler and Sedberg, 1964) has revealed that the production of crossvein defects is due to wing surfaces in the region of the crossvein joining together as in the non vein regions. The

difference between wild-type and crossveinless-like strains is attributable, at the morphological level, to a change in the developmental pattern rather than a removal of a portion of the vein as might be expected. Further, as pointed out before, it appears that the system is controlled by either limiting the amount of "make" produced or the amount used if the amount is in excess as proposed by Milkman (1960a).

This study has recognized a number of responses to temperature shock in a crossveinless-like strain; at least two crossvein restoring responses; two crossvein limiting responses; and a change in sex differences following heat shock. It is yet too soon to determine the function of the major gene which has been identified with this system, but it has been shown that substitution of a wild allele, by the production of heterozygotes, reveals a profound effect of this gene on the responses to temperature shock. It may be that this gene merely provides a broader window for looking at the responses to heat shock which are occurring in the wild-type but are obscured there by the threshold effect. Our purpose now is to summarize what we know about the various responses and attempt to interpret them in the development of the posterior crossvein.

The rapid crossvein restoring response occurs during the majority of the developmental period studied (six hours through 26 hours in development) and has been shown to have a temperature

coefficient (Q_1) of 2.1. The identity of the rapid responses at 14 and 22 hours is not certain because of the difference observed with split treatments, and more experiments are needed to clarify this point. Nevertheless, it appears that the factor being affected in this response is important in the production of crossvein defects.

The slower crossvein restoring response, following prolonged treatment at 22 hours has a Q_1 of at least 1.7.

The reduction in penetrance at 28 hours with temperature shocks up to 20 minutes has not been tested for changes with different temperatures, so that no temperature coefficient is yet available.

Two crossvein limiting responses have been demonstrated, one occurring at 22 hours in development and the other at 28 hours. The crossvein limiting response at 22 hours has a temperature coefficient of 1.9. As of yet no temperature coefficient has been determined for the crossvein limiting reponse at 28 hours. The response at 28 hours strikingly resembles that of Oregon-R at 25 hours in development (Milkman, 1961), for both require a threshold before the crossvein limiting response is observed.

There is yet another interesting point which has been revealed in this study, that is the change in the sex differences after heat shock. This change occurs in age response studies up through 22 hours in pupal development and in dose response with durations as low as five minutes and up through 20 minutes at 40.5°. Not enough

information is available, but it may be that the males have a built in inhibitor, or the females a built in enhancer, which is destroyed by five minute shocks and further treatment causes nearly identical responses in the two sexes.

High temperature coefficients and the corresponding high energies of activation are characteristic of conformational changes in protein due to thermal denaturation. Since we know of no other material in biological systems which have such high temperature coefficients, the responses have been interpreted as such. This, however, does not constitute absolute proof, but the interpretation seems reasonable, and was used by Milkman (1963) in interpreting the various effects produced by temperature shock in wild-type Drosophila.

Milkman's interpretation does not, however, explain all the data now available about posterior crossvein formation, e.g., the recognition of opposing responses to heat shock in Mohler's study with cvl-6b (1965a) and again in this study. It appears that the responses demonstrated in this study occur prior to those described by Milkman (1961, 1962, 1963) for the following reasons: (1) studies by Mohler and Swedberg (1964) and Wong (1966b) have shown that cvl-5 is retarded in stages in wing vein development and temperature-effective period as compared with other cvl strains and with wild-type; and (2) the crossvein limiting response at 28 hours is like that

of wild-type (Oregon-R) at 25 hours in development. If this in fact is valid, i.e., that the responses of this study are prior to those previously described, then they are even more interesting because they may be involved in setting the stage for what occurs latter in development.

Therefore, it is interesting to explore the possibility that the various responses observed may be involved with the control system active in the development of the posterior crossvein. Admittedly, many of the ideas about control of the development of the crossvein are conditioned by the models demonstrated in microbial systems.

It is possible that the rapid crossvein restoring response is affecting a protein molecule which represses the formation of yet another protein necessary for the production of complete crossveins. Thus, changes in the conformation of the protein which is detected early in pupal development derepresses the system and allows the formation of the second protein which is necessary for the production of the complete crossvein. This second protein occurs later in time and is reflected in the variety of responses seen at 22 hours in development, which may be much like those observed by Milkman (1963). Further, the temperature shock at 28 hours is presumably acting upon the second protein and temperature shock at this time destroys the activity of this protein so that complete crossveins

cannot be produced. By this time in development, 28 hours, the effect of the early protein is complete, for by this time short term shocks can no longer produce complete crossveins, so that the function of the early protein is complete at 28 hours. Alternatively, the two proteins may be involved in a reaction sequence leading to the formation of the posterior crossvein. Under this model, change in the conformation of the first protein, i. e., the one demonstrated in the early responses, allows it to assume a more active configuration and therefore it is able to produce more of the material necessary for the second reaction. The later responses could be interpreted the same as in the first model.

Admittedly, both of these models are highly speculative but the data is not inconsistent with such interpretation. Still, several of the responses need further experimentation in order to more precisely characterize them. One of these is the reversal of the crossvein limiting response at 22 hours to a crossvein restoring response. The split treatment at 22 and 22 1/2 hours may allow further clarification of this response, as the problems encountered with long durations, such as death, are minimized by this approach. Another response which needs further experimentation is the lack of protection obtained with the 22 and 22 1/2 hour split treatment; additional experiments with longer intervals may help to elucidate this point. However, it may be that protection is a property of the

early response, indeed, Milkman (1963) concluded that the crossvein making material must exist very early in pupal development as he was able to obtain protection at very early times in development, and this study has shown that the early response is one of crossvein restoration, and it was with the split treatment of 14 and 22 1/2 hours that protection was obtained.

At any rate, this study has recognized an additional component in the formation of the posterior crossvein not previously recognized.

SUMMARY

1. A study of the types and nature of responses to temperature shock was made with a crossveinless-like strain of Drosophila melanogaster.
2. Age response studies revealed three responses at different times in development; a crossvein restoring response at 14 hours in development, a crossvein limiting response at 22 hours in development, and a reduction in penetrance at 28 hours in development.
3. Characterization of the separate responses recognized only one response at 14 hours, rapid crossvein restoration. At 22 hours, three separate responses were found, first, a rapid crossvein restoration with short durations of treatment; next, a crossvein limiting response with intermediate durations, and finally, a crossvein limiting response following long durations of treatment. At 28 hours, two responses were recognized, first, a reduction in penetrance with durations up to 20 minutes, and secondly, a crossvein limiting response following the threshold of 20 minutes.
4. Temperature coefficients were calculated for the various responses. The rapid restoration at 14 hours and 22 hours has a Q_1 of 2.1; the crossvein limiting response at 22 hours has a

Q_1 of 1.9; and the crossvein restoring response following long durations has a Q_1 of at least 1.7. The temperature coefficients at 28 hours have not yet been determined.

5. The high temperature coefficients were interpreted as being due to conformational changes in protein due to thermal denaturation.
6. Rapid crossvein restoration occurs from six hours through 26 hours in development. The identity of the rapid restoration responses at 14 and 22 hours is not certain due to differences in split treatments.
7. Preliminary genetic studies revealed a profound effect of the major gene on the responses to temperature shock. As a first step in studying this effect the major gene was localized to 48.1 on the genetic map of the third chromosome.
8. The results were interpreted as being consistent with either a control mechanism affecting the development of the posterior crossvein, or as steps in a reaction sequence involved in the development of the posterior crossvein.

BIBLIOGRAPHY

Bateman, K. G. 1959. The genetic assimilation of four venation phenocopies. Journal of Genetics 56:443-474.

Beerman, W. 1961. Ein Balbiani-ring als locus einer speicheldrüsenmutation. Chromosoma 12:1-25.

Berendes, H. D., F. M. A. Van Breugel and T. K. H. Holt. 1965. Experimental puffs in salivary gland chromosomes of Drosophila hydei. Chromosoma 16:35-46.

Bridges, C. B. and K. S. Brehme. 1944. The mutants of Drosophila melanogaster. Washington 252 p. (Carnegie Institution of Washington. Publication no. 552)

Eyring, H. and A. E. Stearn. 1939. The application of the theory of absolute reaction rates to proteins. Chemical Reviews 24: 253-270.

Goldschmidt, R. B. 1935. Gen und Ausseneigenschaft (Untersuchen an Drosophila). Zeitschrift für induktive Abstammungs-und Vererbungslehre 69:38-69.

Hadorn, E. 1965. Problems of determination and transdetermination. Brookhaven Symposia in Biology 18:148-161.

Haung, R-c. C. and J. Bonner. 1962. Histone, a suppressor of chromosomal RNA synthesis. Proceedings of the National Academy of Sciences 48:126-1222.

Jacob, F. and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. Journal of Molecular Biology 3: 318-356.

Karlson, P. 1965. Biochemical studies of ecdysone control of chromosome activity. In: Symposium on Hormonal Control of Protein Biosynthesis, Oak Ridge. 1965. Journal of Cellular and Comparative Physiology 66:(Suppl. 1):69-75.

Milkman, R. D. 1960a. The genetic basis of natural variation I. Crossveins in Drosophila melanogaster. Genetics 45:35-48.

Milkman, R. D. 1960b. The genetic basis of natural variation II. Analysis of a polygenic system in Drosophila. Genetics 45: 377-391.

Milkman, R. D. 1961. The genetic basis of natural variation III. Developmental lability and evolutionary potential. Genetics 46:25-38.

Milkman, R. D. 1962. Temperature effects on day old Drosophila pupae. Journal of General Physiology 45:777-779.

Milkman, R. D. 1963. On the mechanism of some temperature effects on Drosophila. Journal of General Physiology 46: 1151-1170.

Milkman, R. D. 1964. The genetic basis of natural variation V. Selection for crossveinless polygnes in new wild strains of Drosophila melanogaster. Genetics 50:625-632.

Milkman, R. D. 1965a. The genetic basis of natural variation VI. Selection of a crossveinless strain of Drosophila by phenocopying at high temperature. Genetics 51:97-99.

Milkman, R. D. 1965b. The genetic basis of natural variation VII. The individuality of polygenic combinations in Drosophila. Genetics 52:789-799.

Milkman, R. D. 1966. The genetic basis of natural variation IX. Comparative studies on cve phenocopy sensitivity in Drosophila. Genetics: in press.

Mohler, J. D. 1965a. The influence of some crossveinless-like genes on the crossveinless phenocopy sensitivity in Drosophila melanogaster. Genetics 51:329-340.

Mohler, J. D. 1965b. Preliminary genetic analysis of crossveinless-like strains of Drosophila melanogaster. Genetics 51:641-651.

Mohler, J. D. 1966. Regulation of gene activity in interacting crossveinless-like genotypes of Drosophila melanogaster. Genetics (accepted for publication 1966).

Mohler, J. D. and G. S. Swedberg. 1964. Wing vein development in crossveinless-like strains of Drosophila melanogaster. Genetics 50:1403-1419.

Mosteller, F. and J. W. Tukey. 1949. The uses and usefulness of binomial probability paper. Journal of the American Statistical Association 44:174-212.

Paigen, K. and R. Ganschow. 1965. Genetic factors in enzyme realization. Brookhaven Symposia in Biology 18:99-115.

Rendel, J. M. 1959. Canalization of the scute phenotype of Drosophila. Evolution 13:425-439.

Rendel, J. M. 1962. The relationship between gene and phenotype. Journal of Theoretical Biology 2:296-308.

Ritossa, F. 1962. A new puffing pattern induced by temperature shock and DNP in Drosophila. Experientia 18:571-573.

Sadler, J. R. and A. Novick. 1965. The properties of repressor and the kinetics of its action. Journal of Molecular Biology 12:305-327.

Waddington, C. H. 1953. Genetic assimilation of an acquired character. Evolution 7:118-126.

Waddington, C. H. 1962. Genetic assimilation. Advances in Genetics 10:257-293.

West, E. S. 1963. Textbook of biophysical chemistry. 3d ed. New York, Macmillan. 435 p.

Wong, P. T. C. 1966a. Temperature-effective periods during crossvein development in crossveinless-like strains of Drosophila melanogaster. Masters thesis. Corvallis, Oregon State University. 61 numb. leaves.

Wong, P. T. C. 1966b. Unpublished research on temperature-effective periods during development in crossveinless-like strains of Drosophila melanogaster. Corvallis, Oregon State University, Dept. of Zoology.

APPENDIX

APPENDIX

Table 1. Dose response of Ona X, Ona II, 5-hi III. 22 hours of age treated with 38.5°.

Duration in minutes	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
5	44	77.3	2.6	31	12.9	1.8
10	40	77.5	2.5	30	26.7	2.0
15	41	53.7	2.2	34	14.7	1.4
20	28	57.1	1.7	27	7.4	1.0
40	42	66.7	3.6	36	22.2	3.6
60	48	72.9	5.5	27	59.3	4.8
80	36	72.2	5.7	37	64.9	4.9
100	30	80.0	6.3	29	62.1	4.0
120	27	100.0	8.1	31	71.0	5.0
140	17	100.0	7.1	21	90.5	4.8
160	1	100.0	5.0	16	68.8	4.4
180	12	83.3	5.6	20	70.0	3.1
Cont.	161	93.8	2.8	145	39.3	1.7

Table 2. Dose response of Ona X, Ona II, 5-hi III. 22 hours of age treated with 39.5°.

Duration in minutes	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
2	31	90.3	2.6	41	46.3	1.4
4	33	63.6	2.2	38	26.3	1.3
6	37	62.2	2.6	31	16.1	1.0
10	39	41.0	1.8	30	3.3	1.0
20	40	70.0	3.8	34	44.1	3.5
30	31	93.5	5.1	35	68.6	4.9
40	41	95.1	6.0	36	72.2	4.9
50	37	97.3	6.3	33	87.9	4.7
60	36	94.4	6.1	29	72.4	4.0
70	34	97.1	6.2	28	60.7	4.2
80	14	92.9	4.8	14	64.3	4.9
90	6	100.0	2.8	5	60.0	4.7
Cont.	150	92.0	2.8	137	44.5	1.5

Table 3. Dose response of Ona X, Ona II, 5-hi III. 22 hours of age treated with 40.5°.

Duration in minutes	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
1	45	93.3	3.1	27	37.0	1.4
2	35	80.0	2.9	33	33.3	1.4
3	39	71.8	2.1	34	14.7	1.8
4	41	41.5	1.9	31	16.1	1.0
5	46	32.6	2.3	28	3.6	1.0
10	33	57.6	3.2	29	17.2	2.2
15	30	66.7	3.8	23	47.8	4.9
20	36	86.1	4.7	32	59.4	4.5
25	31	74.2	4.9	21	57.1	3.3
30	20	60.0	4.3	21	23.8	3.0
35	4	25.0	4.0	3	0.0	0.0
40	1	0.0	0.0	0	---	---
Cont.	156	90.4	3.1	132	50.0	1.7

Table 4. Dose response of Ona X, Ona II, 5-hi III. 22 hours of age treated with 41.5°.

Duration in minutes	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
1	40	75.0	2.5	28	21.4	1.8
2	36	33.3	1.9	33	6.1	1.0
3	38	36.8	2.1	33	3.0	1.0
4	38	34.2	2.4	27	3.7	1.0
5	25	60.0	4.7	32	9.4	2.0
6	24	58.3	2.6	33	24.2	3.6
8	37	89.2	3.7	20	75.0	3.9
10	30	83.3	4.5	23	56.5	4.1
12	21	81.0	4.5	22	63.6	3.6
14	29	82.8	5.5	23	47.8	4.5
16	14	78.6	5.6	22	45.5	2.3
18	15	60.0	4.6	13	30.8	2.5
20	1	0.0	0.0	5	0.0	0.0
Cont.	188	88.3	3.1	144	44.4	1.7

Table 5. Dose response of Ona X, Ona II, 5-hi III. 22 hours of age treated with 42.5°.

Duration in minutes	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
1	37	43.2	1.9	26	11.5	2.0
2	43	37.2	2.3	28	14.3	2.8
3	39	64.1	3.0	28	32.1	3.2
4	26	73.1	3.3	28	75.0	3.7
5	35	80.0	5.6	24	70.8	3.4
6	36	77.9	5.9	24	79.2	5.2
7	29	72.4	4.6	13	61.5	3.5
8	6	33.3	7.0	11	9.1	5.0
9	14	64.3	5.1	17	47.1	3.3
10	11	45.5	4.0	6	16.7	2.0
Cont.	157	89.2	3.1	140	42.9	1.6

Table 6. Dose response of Ona X, Ona II, 5-hi III. 22 hours of age treated with 43.5°.

Duration in minutes	Females			Males		
	n	%	r'_{10}	n	%	r'_{10}
1	39	51.3	2.0	32	0.0	0.0
2	32	59.4	3.3	35	54.3	3.6
3	11	81.8	4.6	19	73.7	4.6
4	5	80.0	5.0	8	37.5	2.7
5	0	---	---	2	100.0	4.0
Cont.	67	97.0	2.8	63	54.0	1.0

Table 7. Dose response of Ona X, Ona II, 5-hi III. 14 hours of age treated with 39.5°.

Duration in minutes	Females				Males			
	n	%	$\bar{r}_{10}$	r'_{10}	n	%	$\bar{r}_{10}$	r'_{10}
2	34	76.5	1.5	2.0	28	7.1	0.1	1.5
4	28	35.7	0.6	1.8	31	3.2	0.0	1.0
6	36	38.9	1.0	2.6	37	2.7	0.1	2.0
10	33	21.2	0.3	1.3	35	0.0	0.0	0.0
15	30	15.2	0.3	2.0	33	3.0	0.0	1.0
20	34	11.8	0.2	1.3	28	0.0	0.0	0.0
Cont.	71	85.9	2.4	2.8	46	43.5	0.4	1.0

Table 8. Dose response of Ona X, Ona II, 5-hi III. 14 hours of age treated with 40.5°.

Duration in minutes	Females				Males			
	n	%	$\bar{r}_{10}$	r'_{10}	n	%	$\bar{r}_{10}$	r'_{10}
1	33	87.9	1.9	2.2	24	33.3	0.8	2.0
2	32	81.3	1.7	2.1	27	18.5	0.2	1.2
3	24	58.3	0.9	1.5	24	4.2	0.1	2.0
4	29	31.0	0.5	1.4	23	8.7	0.1	1.0
5	39	12.8	0.2	1.6	22	0.0	0.0	0.0
10	30	10.0	0.1	1.3	18	0.0	0.0	0.0
15	26	0.0	0.0	0.0	16	0.0	0.0	0.0
Cont.	75	94.7	2.8	3.0	63	44.4	0.9	2.0

Table 9. Dose response of Ona X, Ona II, 5-hi III, 14 hours of age treated with 41.5°.

Duration in minutes	Females				Males			
	n	%	$\bar{r}_{10}$	r'_{10}	n	%	$\bar{r}_{10}$	r'_{10}
1	34	64.7	1.2	1.9	24	16.7	0.4	2.5
2	34	17.7	0.3	1.8	25	4.0	0.1	2.0
3	36	11.1	0.2	1.8	23	0.0	0.0	0.0
4	32	15.6	0.2	1.4	17	0.0	0.0	0.0
6	32	15.6	0.3	1.6	21	0.0	0.0	0.0
8	30	13.3	0.2	1.5	16	0.0	0.0	0.0
10	33	0.0	0.0	0.0	10	0.0	0.0	0.0
Cont.	80	92.5	3.0	3.2	51	31.4	0.6	1.8