A QUANTITATIVE DESCRIPTION OF SOME TEMPERATURE EFFECTS ON DROSOPHILA

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The appearance of the posterior crossvein of the wing of adult *Drosophila* is a delicate measure of physicochemical processes underlying wing vein development during early pupal life. Previous studies of the disturbance of posterior crossvein formation by temperatures from 30° C. to 42.5° C. (Milkman, 1961, 1962, 1963) have led to the outlining of a complex hypothesis which seemed capable of explaining a large number of experimental results (Milkman, 1963). It is the purpose of this paper to cast the hypothesis in a completely quantitative form and to show that the predictions of the model do indeed agree with published and hitherto unpublished experiments.

The experimental background of these studies starts with the basic observation that heat damage to the development of the posterior crossvein is a threshold phenomenon. At temperatures from 39.5° to 41.5° the time to reach a threshold response, or any more severe disturbance, decreases by a one-degree temperature coefficient (Q_1) as large as 2.3. Subthreshold effects are readily revealed by additional heat treatments to consist of a series of related processes which precede the process leading to damage. Nearly threshold pretreatments are definitely damaging as they are additive with a second treatment. Briefer pretreatments, however, would in certain conditions lead to a rapid temperature adaptation which protected the pupa against the damaging effects of additional treatments. In some cases the protection was transient and in others it persisted for many hours. The wealth of effects emerging from these experiments led to the original kinetic scheme (Milkman, 1963). It was suggested that high temperatures induced a sequence of tertiary structure changes in some hypothetical protein required for crossvein formation in the pupa. One could explain the various effects of pretreatments by the properties of the predominant tertiary structure state remaining after the pretreatment. The sole and yet compelling evidence that a protein's tertiary structure was being changed was the very high temperature coefficient of some of the individual steps.

Postulates

The full details of the original scheme can be incorporated into four postulates which may be regarded as the hypothesis to be demonstrated by this paper:

1. Normal development of the posterior crossvein requires a protein A within a few hours after a biological age equivalent to 25 hours of pupal development at 23° C.

2. At elevated temperatures A is changed to states B, C, C′, D, E, F and G as in the reaction sequence below:

$$\begin{array}{ccccccccc} A & \rightleftharpoons & B & \rightarrow & D & \rightleftharpoons & E & \rightarrow & F \rightarrow G \\ & & \uparrow\downarrow & & \downarrow & & & & \\ & & C & \rightarrow & C' & & & & \end{array}$$

The individual steps of the conversion, represented by arrows, are first order in the reactant and may be assigned rate constants and temperature coefficients.

3. The algebraic sum of the amounts of protein in all the states except G linearly determines the ultimate length of the posterior crossvein within a defined range. Thus defects result from treatments which produce G, the terminal inactive state.

4. The adjustable parameters of the model: rate constants, temperature coefficients, initial amount of A, and the function relating posterior crossvein length to the sum of the effective states, are themselves functions of the age, raising temperature, sex, and genome of the organism.

Materials and Methods

Because of postulates 1 and 4, in this paper we evaluate the parameters only for female Oregon R *Drosophila melanogaster* 25-hour pupae raised at 23° C. This age happens to be the time of maximum heat sensitivity of posterior crossvein formation (Milkman, 1961, 1962).

The equations of the model were solved on a ten-amplifier Donner 3500 analog computer using standard techniques for linear systems. Initial conditions were set with a Tektronix 161 pulse generator. The time course of the solutions was photographed on Polaroid projection film from the face of a Tektronix 502 oscilloscope. The curves were enlarged on graph paper by tracing the projected image under an enlarger. We often took advantage of the additivity of solutions of linear systems when curves for experiments involving split treatments were to be calculated. In a two-temperature experiment, for example, the quantities of all the intermediates after the first treatment were calculated in a straightforward manner and then the separately calculated fates of each intermediate in the second treatment were added to get the final solution. The maximum error was less than 5%. This error will be seen to correspond to an error of less than one crossvein rating unit in the predictions.

The fruit flies used in these experiments were a highly inbred strain of Oregon R *Drosophila melanogaster* raised by standard techniques in an incubator at 23°. Pupae were placed in shell vials within one hour after puparium formation, to be aged in a 23° regulated water bath. Depending on the duration of treatment, high temperature heat treatments were started sometime after the 24th hour of development and were completed by the 26th hour. Unfortunately, even during this period, the response of the flies changes sufficiently rapidly to affect the agreement between experiment and predictions (Milkman, 1961, 1962). Short experiments whose total treatment could be given close to the 25th hour were therefore considered to be of the greatest importance. The high temperature water baths were regulated to within ±0.05°, but their absolute temperature can have differed by ±0.15° from one year's experiments to the next. This variation of temperature would lead to a variation in experimental data approximately equivalent to a 10% change in the time axis. For all short treatments the pupae were in a teabag. Teabags or

vials were used in longer experiments. While the results of identical treatments in teabags and vials are different at times, we have made no effort to distinguish them in this paper. We have also ignored any possible warm-up time for glass vial treatments. At least five days later, after the flies had emerged, their posterior crossveins were rated on a scale from zero (normal) to twelve (none remaining) according to the total length of posterior crossvein remaining on the two wings and regardless of how the remaining fragments were distributed. The ratings (r) of about 50 flies were averaged for each data point. The details of these methods have been published before (Milkman, 1961, 1962). Almost all of the data used here have been published already (Milkman, 1962, 1963; Milkman and Hille, 1966).

The figures of this paper contain points, lines, and a summary of the temperature sequences used. The points are always experimentally determined data points referring to large numbers of flies. The lines are theoretically calculated using the explicitly stated postulates, the rate constants, and the conversion factor between G units and crossvein rating units. There are no additional adjustable parameters, so that to the degree that the lines and dots in all examples agree, the unified scheme is satisfactory. The temperature sequences have been indicated diagrammatically by a series of rectangular steps. If the duration at a particular temperature is variable, the horizontal line has been dotted; otherwise the line is solid.

Results

To explain how our hypothesis fits experimental data we must first examine certain mathematical properties of the model system without reference to any experiments. According to postulate 2, first-order differential equations for the rate of change of each state may be written in the conventional form of chemical kinetics:

$$\frac{dA}{dt} = -k_{AB}A + k_{BA}B$$

$$\frac{dB}{dt} = k_{AB}A - (k_{BA} + k_{BC} + k_{BD})B + K_{CB}C$$

$$\frac{dC}{dt} = k_{BC}B - (k_{CB} + k_{CC'})C$$

$$\frac{dC'}{dt} = k_{CC'}C + k_{DC'}D$$

$$\frac{dD}{dt} = k_{BD}B - (k_{DC'} + k_{DE})D + k_{ED}E$$

$$\frac{dE}{dt} = k_{DE}D - (k_{ED} + k_{EF})E$$

$$\frac{dF}{dt} = k_{EF}E - k_{FG}F$$

$$\frac{dG}{dt} = k_{FG}F$$

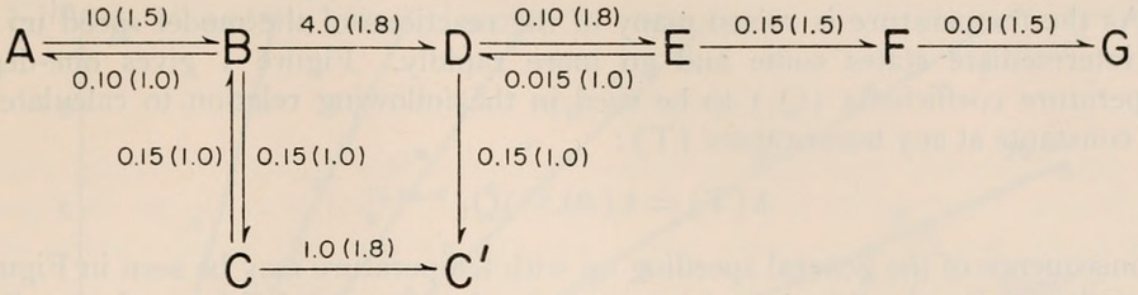


FIGURE 1. The parameters of the model. The first number by each arrow is the first order rate constant in min.$^{-1}$ for that step. The second number, in parentheses, is the one-degree temperature coefficient, Q_1, of the rate constant.

This linear system of simultaneous differential equations may be solved analytically to give an answer in closed form, but this type of solution is impractical because of the tedious calculations required to make quantitative predictions. For this reason we used an analog computer, an ideal instrument for linear systems, to see solutions displayed directly on an oscilloscope screen where the effect of any adjustment in the parameters was immediately observable.

Figure 1 shows the rate constants estimated for 40.5°. Figure 2 is a photograph of a solution using these rate constants and starting with 100 units of A and zero units of all other states. In a fraction of a minute A is gone. The subsequent intermediates rise to a peak and fall again until by 25 minutes the only reaction proceeding at a significant rate is the conversion of F to G. It will be a helpful rule to remember that even in this complicated system most reactions will have half-times given in minutes by 0.7 times the reciprocal of the rate constant k. In the presentation we shall speak loosely of the time when a certain reaction is complete. We shall really refer to the time when the reaction is 80% to 90% complete.

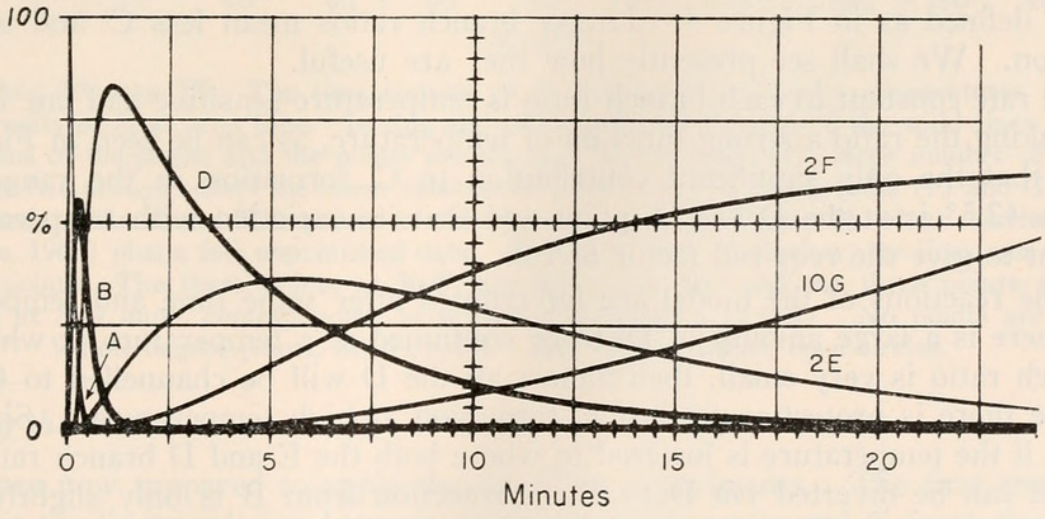


FIGURE 2. The time courses of the states of the model at 40.5° photographed from the face of the oscilloscope. The solution was started with 100 units of A (four boxes on the reticle) and zero units of all other states. As indicated, some of the curves are recorded at higher gain for better resolution. C formation is negligible. C′ formation is 60 units at 20 minutes. Neither state is recorded here. At other temperatures the temporal relationships are different.

As the temperature is raised many of the reactions of the model speed up and the intermediate states come and go more rapidly. Figure 1 gives one-degree temperature coefficients (Q_1) to be used in the following relation to calculate the rate constants at any temperature (T):

$$k(T) = k(40.5°)Q_1^{\,T-40.5°}$$

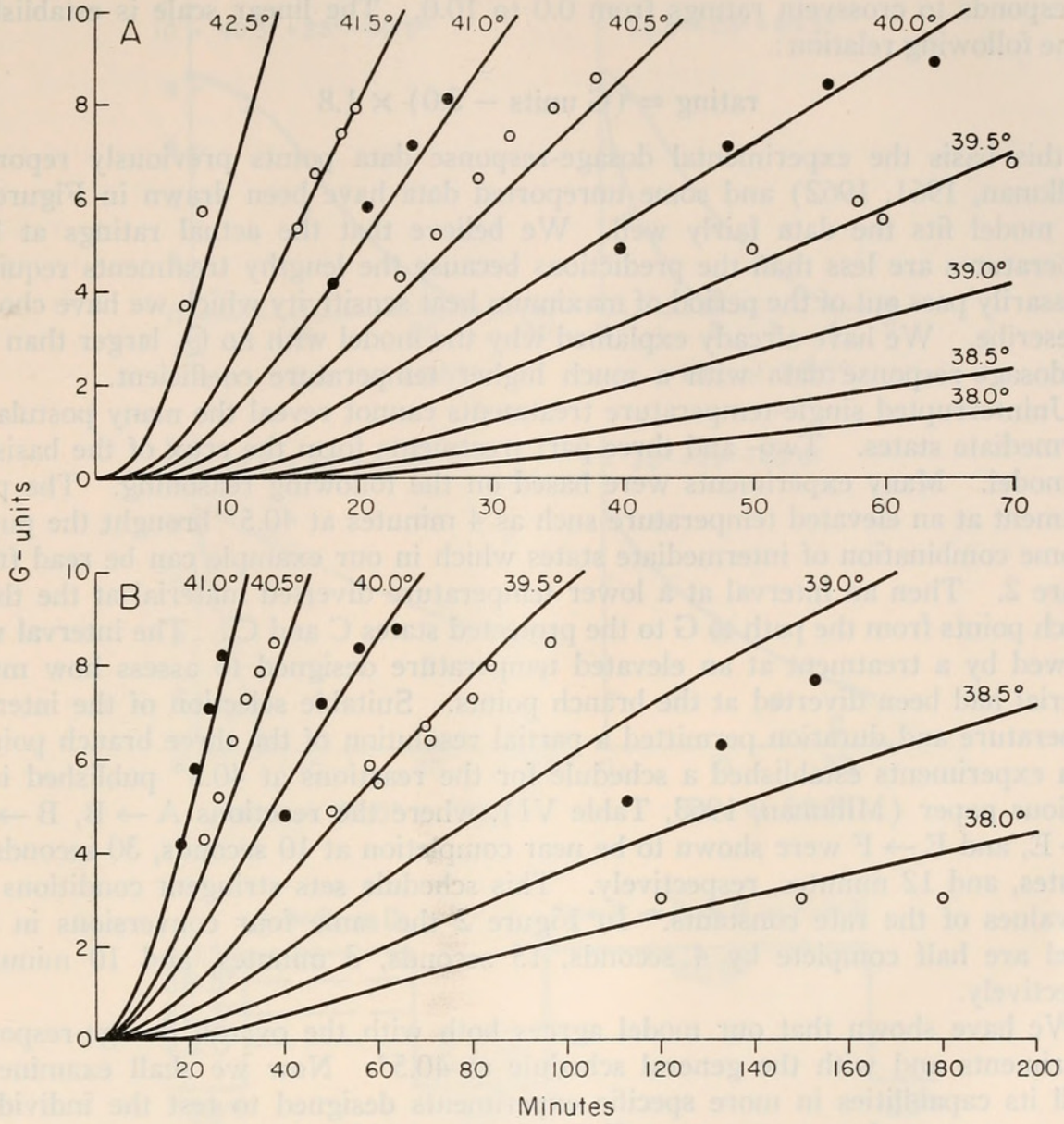
A consequence of the general speeding up with temperature may be seen in Figure 3 where the family of solid curves represents the time course of G formation at different temperatures. These curves behave as though they were generated by a process having a Q_1 greater than 2.0. This is surprising at first because Q_{1FG} is only 1.5 and no other reaction has a Q_1 higher than 1.8. The extra multiplicative factor of more than 1.3 is explained as follows:

There are two terminal states in the model, C′ and G. In general we may say that the more C′ is made, the less material can ever become G and the slower is G formation at any time. Thus we could call C′ a "protected" state because its formation prevents the formation of G, the damaged state. At 40.5° the pattern of rates of the reaction favors the production of C′ over the production of G. At higher temperatures there is a different pattern and more material is destined for G. It is this temperature-dependence of the amount of G to be formed that provides the additional factor which accounts for the very high Q_1 of G formation.

The amount of C′ formed depends on four important pathways of protection: directly from D, directly from C, indirectly from B *via* C, and indirectly from E *via* D. Each of these assumes importance at different temperatures. Let us first consider the direct route from D. Some D goes to E and some to C′. Thus D may be said to be at a "branch point." The velocities of E and C′ formation from D are in the same proportion as the rate constants k_{DE} and $k_{DC'}$. We therefore define the concept of "D branch ratio" as k_{DE} divided by $k_{DE} + k_{DC'}$. In words it is the fraction of D going to E, but we must bear in mind that some of the E produced may be returning to D at the same time. Similarly the B, C, and E branch ratios may be defined as in Figure 4. Larger branch ratios mean less C′ and more G formation. We shall see presently how they are useful.

One rate constant in each branch ratio is temperature-sensitive and one insensitive, making the ratio a strong function of temperature, as can be seen in Figure 4. Notice that the only significant contribution to C′ formation in the range from 38.5° to 42.5° is at the D branch point and that its variation with temperature is sufficient to give the required factor of 1.3.

If the reactions of the model are interrupted after some time and temperature when there is a large amount of D, to be continued at a temperature at which the D branch ratio is very small, then almost all the D will be channelled to C′. In this case there is protection against G formation at high temperatures. Similarly with E, if the temperature is lowered to where both the E and D branch ratios are small, E can be diverted *via* D to C′. Protection from B is only slightly more complex in that the B and C branch ratios are never simultaneously very low. Therefore a low temperature is first required to make C followed by a high temperature to make C′. Because C readily forms C′ at high temperatures, we shall also use "protected" to describe C. Thus the curves of Figure 4 are useful for selecting temperatures at which particular pathways of C′ formation will be operant.



FIGURES 3A AND 3B. The time courses of G formation at various temperatures starting with 100 units of A at zero time. In this and all figures after Figure 4 the solid lines are the expectations of the model and the points are the average response of a large number of female flies. The two always reflect the linear relation between crossvein rating units (r) and G formation derived in the text. The points in this figure are from Figure 1b of an earlier paper (Milkman, 1962) plus a few unpublished data. Except at 39.0° and below, the lines run close to the data points. The three points at 39.0° are below the line and the three points at 38.5° are down at 3 G units, corresponding to no crossvein defects ($r = 0$). No points are shown from 38.0°. Whole degree points, filled circles. Half degree points, open circles.

Applying the model

We are now prepared to apply the model to experiments. The first step is to determine the linear relation between crossvein rating units and G formation as in postulate 3. We shall choose the original (Milkman, 1961) dosage-response experiment at 40.5° as our standard. The threshold for defect production was around 20 minutes and average ratings of 10.0 were produced by 40 minutes. Thus the range of G values from 20 to 40 minutes, namely from 3.0 units to 8.5 units,

corresponds to crossvein ratings from 0.0 to 10.0. The linear scale is established by the following relation:

$$\text{rating} = (\text{G units} - 3.0) \times 1.8$$

On this basis the experimental dosage-response data points previously reported (Milkman, 1961, 1962) and some unreported data have been drawn in Figure 3. The model fits the data fairly well. We believe that the actual ratings at low temperatures are less than the predictions because the lengthy treatments required necessarily pass out of the period of maximum heat sensitivity which we have chosen to describe. We have already explained why the model with no Q_1 larger than 1.8 fits dosage-response data with a much higher temperature coefficient.

Uninterrupted single-temperature treatments cannot reveal the many postulated intermediate states. Two- and three-part treatments form the crux of the basis of the model. Many experiments were based on the following reasoning. The pre-treatment at an elevated temperature such as 4 minutes at 40.5° brought the pupae to some combination of intermediate states which in our example can be read from Figure 2. Then an interval at a lower temperature diverted material at the three branch points from the path to G to the protected states C and C′. The interval was followed by a treatment at an elevated temperature designed to assess how much material had been diverted at the branch points. Suitable selection of the interval temperature and duration permitted a partial resolution of the three branch points. Such experiments established a schedule for the reactions at 40.5° published in a previous paper (Milkman, 1963, Table VI), where the reactions $A \rightarrow B$, $B \rightarrow D$, $D \rightarrow E$, and $E \rightarrow F$ were shown to be near completion at 10 seconds, 30 seconds, 5 minutes, and 12 minutes, respectively. This schedule sets stringent conditions on the values of the rate constants. In Figure 2 the same four conversions in the model are half complete by 4 seconds, 15 seconds, 3 minutes, and 10 minutes, respectively.

We have shown that our model agrees both with the overall dosage-response experiments and with the general schedule at 40.5°. Now we shall examine in detail its capabilities in more specific experiments designed to test the individual rate constants and temperature coefficients over a wide range of temperatures.

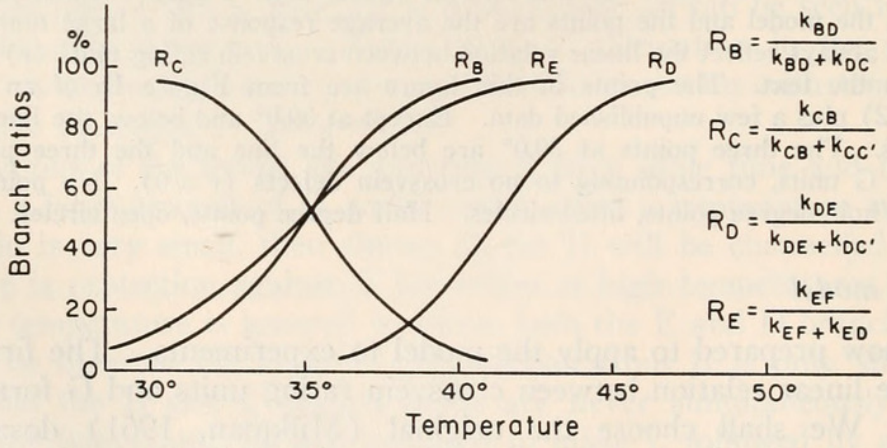


FIGURE 4. The branch ratios as a function of temperature. The ratios are defined on the right and explained in the text. In general, the larger the ratio at a branch point, the more material can get from that branch point to G.

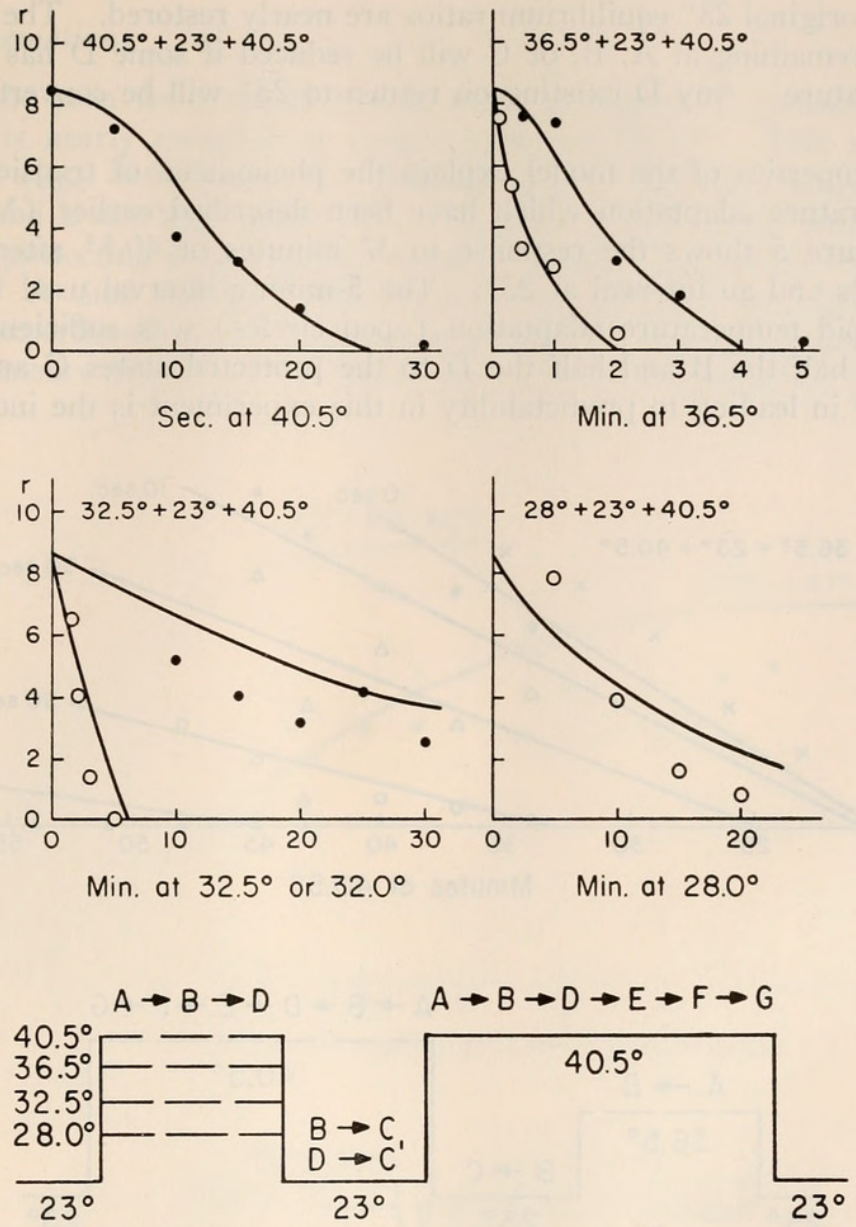


FIGURE 5. Transient and lasting rapid temperature adaptation at different temperatures. The pretreatment temperature and duration are indicated. Treatments were 37 minutes at 40.5°. Transient adaptation (open circles) was achieved with a 5-minute interval at 23°. These data from previously published experiments (Milkman, 1963, Table II). Lasting adaptation (solid circles) used a 60- to 90-minute interval, depending on duration of pretreatment (at 24 hours). These data are unpublished but similar to published experiments (Milkman, 1963, Table IV) where pretreatments started at 21 hours. The ratings decrease as protection increases.

B, C, and D formation

The first three states are normally in an equilibrium mixture of 75% A, 6% B, and 19% C at 23°. When the temperature is raised, k_{AB} is increased, more A goes to B, and the equilibrium shifts, B and C becoming re-equilibrated in about 10 minutes. If the temperature is so high that the B branch ratio is large, then most of the B will continue to D, never to equilibrate with C. Returning the temperature to 23° at any moment will let the B → C reaction equilibrate to one part B to three parts C in 10 minutes. At the same time B is returning to A until after 40

minutes the original 23° equilibrium ratios are nearly restored. The actual amount of material remaining at A, B, or C will be reduced if some D has formed at the high temperature. Any D existing on return to 23° will be converted to C′ in 10 minutes.

These properties of the model explain the phenomena of transient and lasting rapid temperature adaptation which have been described earlier (Milkman, 1961, 1962). Figure 5 shows the response to 37 minutes of 40.5° after the indicated pretreatments and an interval at 23°. The 5-minute interval used to demonstrate transient rapid temperature adaptation (open circles) was sufficient to bring approximately half the B and half the D to the protected states C and C′. As the major factor in leading to predictability in this experiment is the increased A → B

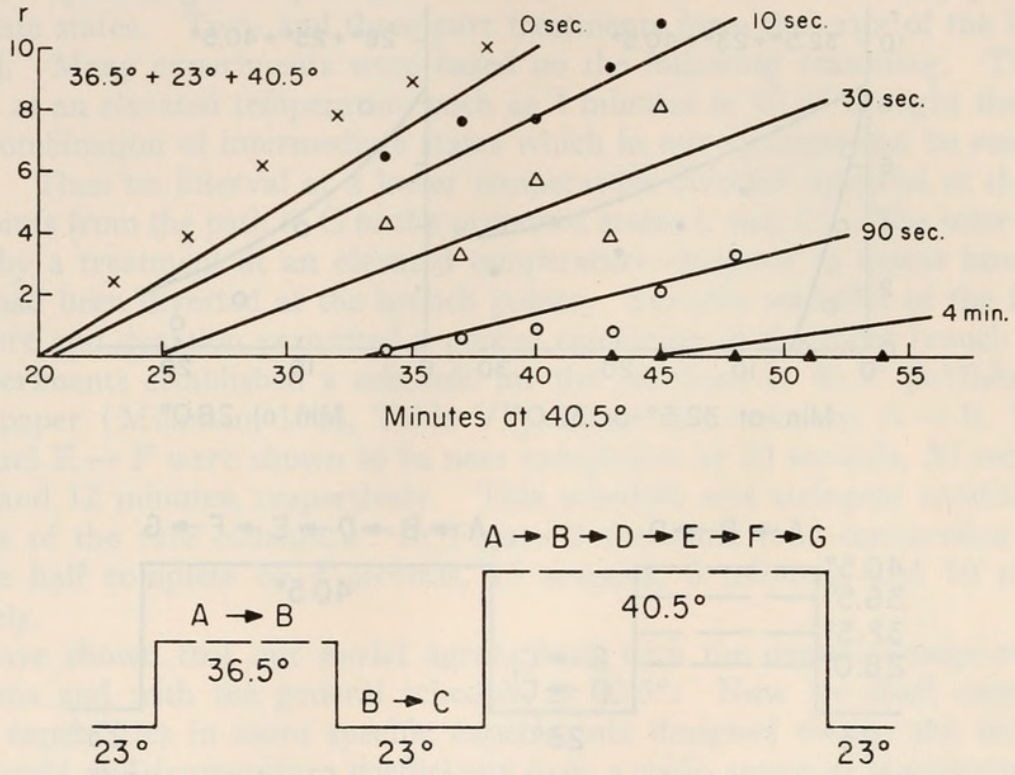


FIGURE 6. Transient rapid temperature adaptation. Dosage-response at 40.5° following pretreatments at 36.5° with a 5-minute interval at 23°. As A is converted to B, the 23° interval diverts more B to a protected state. The protection is manifested as a change in both intercept and slope. Unpublished data.

reaction, we take the agreement of the data (open circles) with the theory (curves) over an 8.5° range to confirm our choice of k_{AB} and Q_{1AB}. The long interval used to demonstrate lasting rapid temperature adaptation (solid circles) was sufficient completely to protect D as C′ and to reverse the formation of B and the protected state C by re-establishing the 23° equilibrium ratios. The agreement between data and theory over 8° confirms the choice of k_{BD} and Q_{1BD}. A second kind of experiment showing that transient rapid temperature adaptation leads to the predicted lowering of the 40.5° dosage-response in slope as well as response to 37 minutes is illustrated in Figure 6. This experiment suggests that rather than just creating a delay in G production, protection diverts material from the pathway to G as we have postulated.

E and F formation

As can be seen from the D branch ratio in Figure 4, protection of D by C′ formation is nearly complete at temperatures below 38°. This step takes about 10 minutes ($k_{DC'} = 0.15$ min.$^{-1}$). Protection of E *via* D formation is slower ($k_{ED} = 0.015$) and is not large above 35°. Therefore a 10-minute interval at 37.5°, by selectively protecting D and not E, serves to measure the time course of the D to E transition. A time course of this transition is recorded in Figure 2. Figure 7 shows an experiment where a 10-minute interval at 37.5° was intercalated between 40.5° treatments totalling 32 minutes (open circles) or between 41.5° treatments

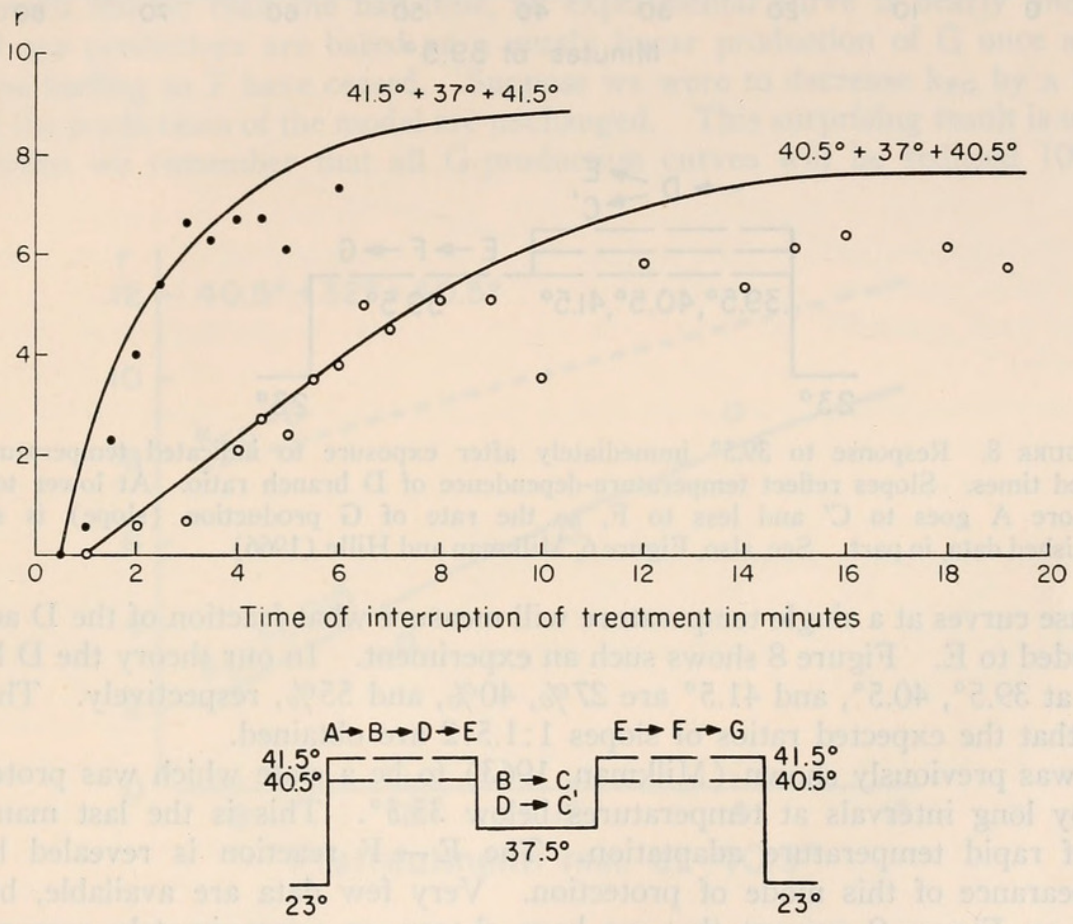


FIGURE 7. The disappearance of D. The response to a 20-minute treatment at 41.5° (solid circles) or a 32-minute treatment at 40.5° (open circles), each with a 10-minute interval at 37.5° intercalated at one of various times (time of interruption). As D is converted to E, the interval at 37.5° becomes less effective in lowering the rating. Data from Figure 3 of Milkman and Hille (1966).

totalling 20 minutes (solid circles). The time and rate of decrease of protectability as D goes to E confirms our choice of k_{DE} and Q_{1DE}.

Because the preceding experiment covered only a 1° range, it is desirable to determine Q_{1DE} over a wider range. This can be done by designing experiments to test the D branch ratio at different temperatures, for, as we have discussed, the temperature dependence of this ratio is a consequence of Q_{1DE}. The branch point ratio may be measured by first exposing pupae to different high temperatures until all the D has been converted to E and C′. Then the slopes of subsequent dosage-

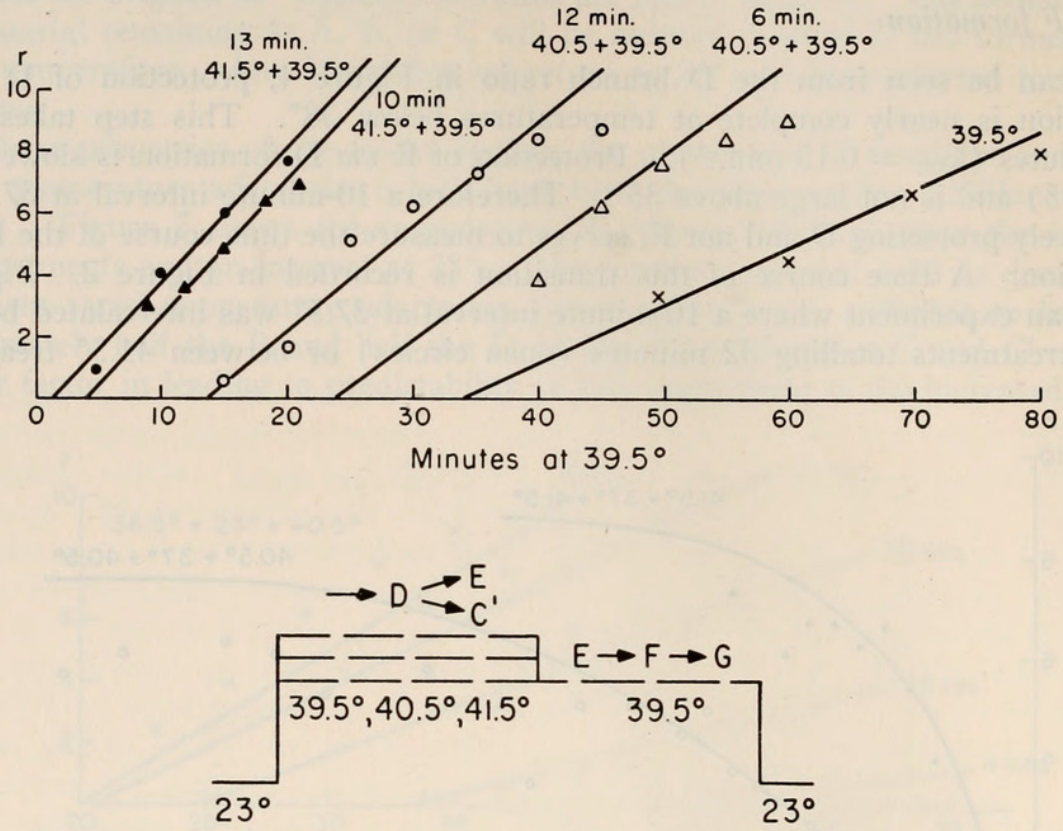


FIGURE 8. Response to 39.5° immediately after exposure to indicated temperatures for indicated times. Slopes reflect temperature-dependence of D branch ratio. At lower temperature more A goes to C′ and less to F, so the rate of G production (slope) is smaller. Unpublished data, in part. See, also, Figure 6, Milkman and Hille (1966).

response curves at a single temperature will measure what fraction of the D actually proceeded to E. Figure 8 shows such an experiment. In our theory the D branch ratios at 39.5°, 40.5°, and 41.5° are 27%, 40%, and 55%, respectively. The data show that the expected ratios of slopes 1:1.5:2 are obtained.

E was previously shown (Milkman, 1963) to be a state which was protectable only by long intervals at temperatures below 35.5°. This is the last manifestation of rapid temperature adaptation. The E → F reaction is revealed by the disappearance of this mode of protection. Very few data are available, but the points on Figure 9 suggest that we have chosen an approximately correct time course. The dashed curve in the figure shows the consequence of removing the reaction which protects E ($k_{ED} = 0$). The difference between this line and the points shows the contribution of E-protection. The solid line is the expectation from the model as it stands. We must now determine Q_{1EF}. As with Q_{1DE} for the D branch ratio, Q_{1EF} governs the temperature dependence of the E branch ratio. If we take Q_{1ED}, k_{ED}, and k_{EF} at their postulated values and try three values: 1.4, 1.5, and 1.7 for Q_{1EF}, we find that the E branch point ratios fall to 50% at 33.5°, 35.0°, and 36.3°, respectively. At present our choice is 1.5 simply to have E-protection below 35.5°.

G formation

After 25 minutes at 40.5° the only reaction still proceeding is the conversion of F to G. As has been illustrated (Milkman, 1963), this is the best time to

measure Q_{1FG}. The curves in Figure 10 show that 1.5 is a good temperature coefficient over a 6° range. It was this unequivocal demonstration of a Q_{1FG} of 1.5 which forced us to seek a further explanation of the 2.3 temperature coefficient of pure dosage-response curves (Fig. 3). A multiplicative factor was discovered in the effect of the D branch point, as we have proven by the experiment of Figure 8. In Figure 3 we saw that Q_{1FG} and the factor from the D branch point are sufficient to explain the temperature dependence of the data.

So far we have not discussed how we chose k_{FG}. At 40.5° the estimated half-time of this reaction is 70 minutes. None of our experiments was long enough to reach the half-time of the F to G reaction at any temperature. Recall that at times much shorter than the half-time, an experimental curve is nearly linear so that all our predictions are based on a nearly linear production of G once all the reactions leading to F have ceased. Suppose we were to decrease k_{FG} by a factor of 100: the predictions of the model are unchanged. This surprising result is understood when we remember that all G-production curves will be reduced 100-fold

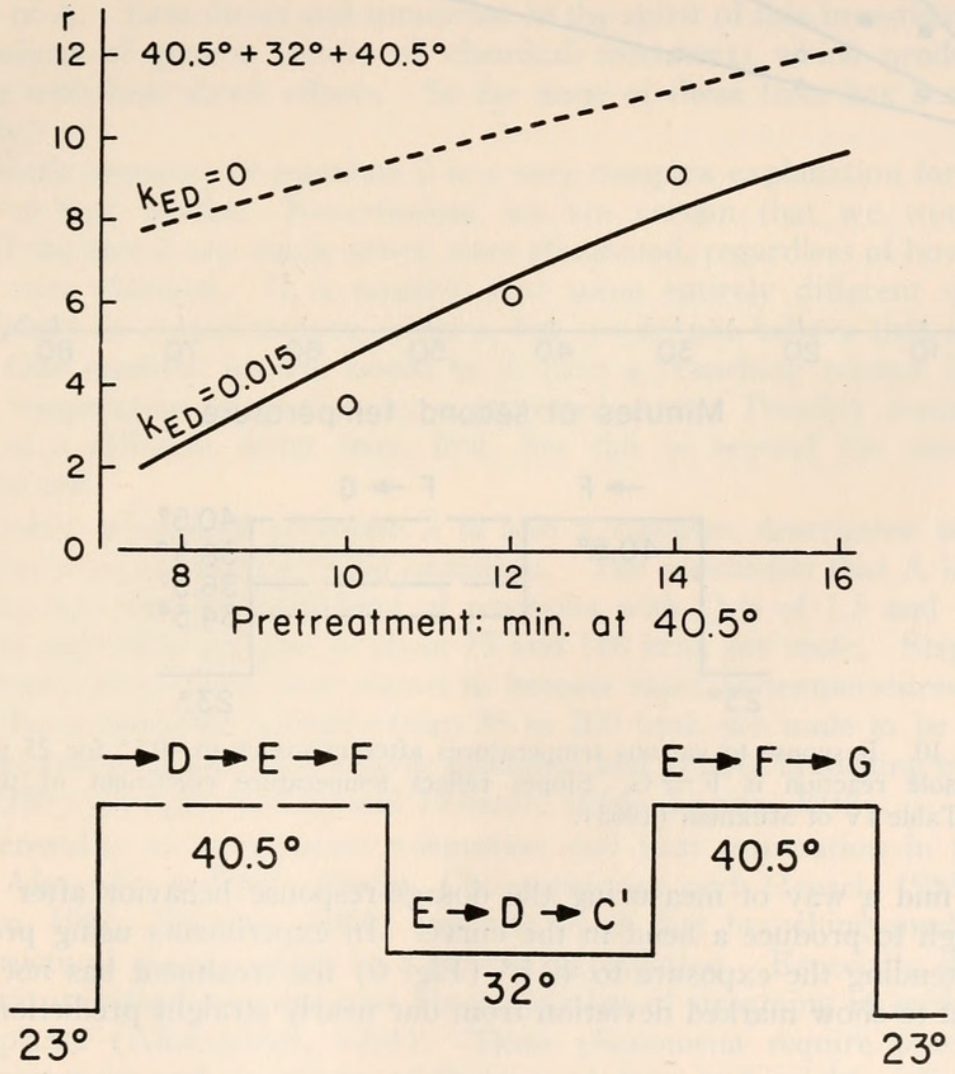


FIGURE 9. Response to 25 minutes at 40.5° after stated pretreatment and 1 hour at 32°. Rapid rise reflects in part the disappearance of E, whose protectability depends on the reversal of the D → E reaction. Were this reaction irreversible, the dashed line would represent the prediction.

and hence the values of G selected to correspond to posterior crossvein ratings from 0 to 10 will also be reduced. For this reason any choice of k_{FG} smaller than the present one will give equally satisfactory predictions. If we increased k_{FG} 3-fold, the 40.5° half-time would be reduced to 23 minutes, and all the dosage-response productions of Figure 1 would curve noticeably. As the agreement would no longer be satisfactory, we cannot increase k_{FG}. Thus our selection of 0.01 for k_{FG} is the maximum permissible. The arbitrariness of this choice could be eliminated if

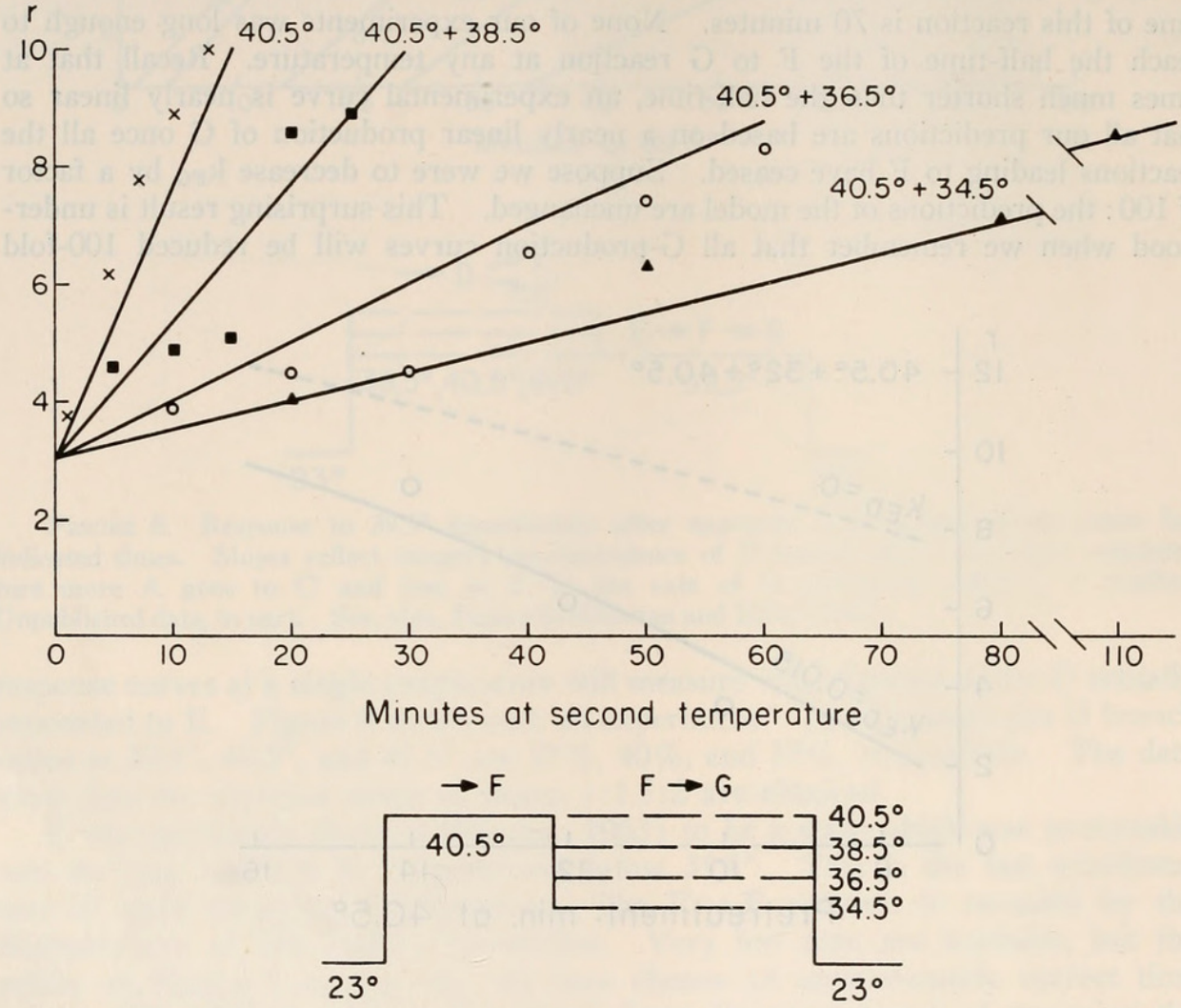


FIGURE 10. Response to various temperatures after exposure to 40.5° for 25 minutes. At this time, sole reaction is F → G. Slopes reflect temperature coefficient of this reaction. Data from Table IV of Milkman (1963).

we could find a way of measuring the dosage-response behavior after treatments long enough to produce a bend in the curve. In experiments using protection to permit extending the exposure to 40.5° (Fig. 6) the treatment has not been long enough yet to show marked deviation from our nearly straight prediction.

The temperature-insensitive reactions

The five reactions with a Q_1 of 1.0 are primarily concerned with the different kinds of temperature adaptation. Their rates can be determined from the length

of the low temperature interval required to achieve a certain amount of protection. As the interval temperature from low temperatures up to 34° does not affect the rate of protection we have chosen Q_1's of 1.0. Experiments depending on some of these relations have been published (Milkman, 1963, Tables VIII and IX, Figure 1). A more thorough demonstration appears in another paper (Milkman and Hille, 1966). In new experiments protection of E is found to be more rapid in the second 10 minutes of interval than in the first. It is because of this lag in E protection that we have chosen to let E revert to D before reaching a protected state, rather than having E form a protected state directly, as has been suggested before (Milkman, 1963).

Discussion

We shall reconsider the four postulates of the model. The existence of the required protein A of postulate 1 is known only through defects produced after heat shocks. It would be desirable to demonstrate A in some other manner. Most meaningful of all would be the chemical isolation of a substance with the properties of A. Less direct and somewhat in the spirit of this investigation would be the analysis of genetic factors or chemical treatments which produce effects interacting with heat shock effects. So far none of these lines has been pursued successfully.

The kinetic sequence of postulate 2 is a very complex explanation for responses of pupae to heat shocks. Nevertheless, we are certain that we would fail to describe all the data if any single arrow were eliminated, regardless of how the other constants were changed. It is possible that some entirely different network of reactions could be as satisfactory as ours, but we do not believe that it could be simpler. One essential feature would be to have a branching scheme in order to get rapid temperature adaptation by a protected state. Possibly some reactions could be of a different order from first, but this is beyond the range of our computer to test.

The kinetic scheme of postulate 2 is also a complex description of the heat denaturation of a protein in a living organism. The conclusion that A is a protein rests on the now firm establishment of reactions with Q_1's of 1.5 and 1.8, corresponding to activation energies of about 75 and 110 kcal. per mole. Steps in other protein denaturations have been shown to become rapid at temperatures from 40° to 60°, to have activation energies from 35 to 200 kcal. per mole to be reversible, to have temperature-insensitive reverse reactions, and to have branching mechanisms (Chase, 1950; Johnson, Eyring and Polissar, 1954; Kunitz, 1948).

The generality of temperature adaptation and heat prostration in plants and animals (Alexandrov, 1964; Precht, Christophersen and Hensel, 1955; Prosser and Brown, 1961; Ushakov, 1964) suggests to us that branching mechanisms of protein structural change might be a general explanation. Especially striking are the temperature adaptation and heat immobilization of streaming in epidermal cells of many plants (Alexandrov, 1964). These phenomena require practically the same temperatures and durations as those used here and might well yield to a similar analysis.

Postulate 3 is striking because it assumes that many tertiary structure states of a protein have full biological activity. In the experiments discussed, only four states

remained in significant quantity by the 26th hour of pupal development: A, C′, F, and G. In the present scheme D could never remain longer than 10 minutes and E never longer than 60 minutes after treatment, each going to C′. B and C could be maintained in high equilibrium concentration by keeping the pupae at the temperatures around 30°. Undoubtedly the shift of the A-B-C equilibrium has an important role in making pupae whose entire pupal life was spent at a higher temperature more resistant to temperature effects. At any rate we have shown that A, C′ and F are active states in posterior crossvein development.

The relationship between crossvein formation and G production shows the extreme sensitivity of this developmental process to changes in the amount of active protein. Defects follow on a 3% (or less, depending on the choice of k_{FG}) loss of active substance. This is not to say that A is a factor required only in posterior crossvein development. It might well be common and essential to many other processes whose sensitivity to small concentration changes is negligible. Indeed, it seems to be essential for life in that flies never live to achieve expected average ratings of 11 and 12 (about 9 G units). Other causes of heat death are also operating, so that in some kinds of heat experiments viability was zero even with very little G production. We should say also that other causes of posterior crossvein disturbance are also operating because in very long treatments at 36° to 38°, defects are produced when there has been almost no G production (Milkman, 1961, 1962). These phenomena remain unexplained. We find it remarkable that the wide range of temperature effects treated here can be described by the fate of a single substance so that the existence of other processes comes as no surprise.

The last postulate, saying that the parameters of the model are functions of the age, sex, genome, etc., has been adequately documented (Milkman, 1961, 1962). It opens the road to studying the development of protein A, its denaturation properties, and its translation into posterior crossvein. Hopefully by the time these properties are known we will also know protein A's chemical and developmental function.

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Summary

1. A complex array of high temperature effects on *Drosophila melanogaster* pupae is described in terms of a quantitative hypothesis. A branched series of reactions, first order in the reactant, provides a unifying basis for a set of adaptational, morphogenetic, and lethal effects.

2. The temperature coefficients of some of the reactions suggest that they may be specific, serial tertiary structure changes in an otherwise undescribed protein.

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