The nerve growth–stimulating effects of the intact tumor, when growing in close proximity to a sensory ganglion, are compared in Plate II, Figures 5–8, with the growth-stimulating effects of extracts obtained from these tumors.

Our investigations are now directed toward (a) the further elucidation of the nature of the active material, (b) the duplication of the effect of the growing tumor in the living embryo with the active material isolated from the tumors, and (c) an examination of the metabolic response of the nerve cells under the influence of the growth-promoting agent.

[1] E. D. Bueker, *Anat. Record*, **102**, 369–390, 1948; R. Levi-Montalcini and V. Hamburger, *J. Exptl. Zool.*, **116**, 321–362, 1951; R. Levi-Montalcini, *Ann. N.Y. Acad. Sci.*, **55**, 330–343, 1952.

[2] R. Levi-Montalcini and V. Hamburger, *J. Exptl. Zool.*, **123**, 233–288, 1953.

[3] R. Levi-Montalcini, H. Meyer, and V. Hamburger, *Cancer Research*, **14**, 49–57, 1954.

[4] W. C. Schneider, *J. Biol. Chem.*, **176**, 259–266, 1948.

[5] S. S. Cohen, *J. Biol. Chem.*, **168**, 511–526, 1947; H. Von Euler and L. Heller, *Arkiv Kemi*, **26A**, No. 10, 1–16, 1948; R. J. Kutsky, *Proc. Soc. Exptl. Biol. Med.*, **83**, 390–395, 1948.

[6] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265–275, 1951.

[7] W. C. Schneider, *J. Biol. Chem.*, **161**, 293–303, 1945.

ON TEMPERATURE INDEPENDENCE IN THE CLOCK SYSTEM CONTROLLING EMERGENCE TIME IN DROSOPHILA*, †

By Colin S. Pittendrigh

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

Clocks, processes measuring absolute time, occur in living organisms. Recently they have attracted interest, especially following the demonstration by Kramer[1] and Pardi and Papi[2] of the celestial navigation performed by birds and amphipods. The present paper is concerned with the clock system controlling the time at which *Drosophila* adults emerge from puparia. The timing of this event has attracted several earlier workers, especially Kalmus[3, 4, 5] and Bünning.[6] Kalmus' account of the temperature relations of the *Drosophila* clock, which is not confirmed here, formed an essential foundation for the present study and led to its being undertaken.

The temperature relations of organic clocks are of obvious interest. If a clock is to provide information involved in controlling important functions, then clearly it must be reasonably reliable. Attention is then focused on how reliable timing can be effected by poikilotherms, like gammarids and flies, in natural environments that are characterized by violent daily temperature variations. A temperature-dependent clock will guarantee only mistiming. Unlikely as it may seem on physical grounds, the biological prerequisite of temperature independence has been achieved by poikilotherms. This was shown more than twenty years ago, in a classical study of the bee's "time-sense," by Wahl.[7] Much more recently, Brown[8] has demonstrated temperature independence in the clocks controlling daily and tidal periodicities in *Uca* and *Venus*.

On the basis of two brief and data-less papers by Kalmus[4,5] the *Drosophila* case has stood out as one in which the clock system is temperature dependent. Experiments described here were undertaken to confirm Kalmus' account. The results indicate a more complex system than he suggested: part of it is, as Kalmus noted, temperature dependent and subject to regulation, though not in the way he claimed; but an all-important part of the system, previously overlooked, is essentially temperature independent.

Methods.—Data given here were all obtained using a single strain of *Drosophila pseudoobscura* Sturtevant collected in 1950 from Mather, California, in the Sierra Nevada, by Professor Th. Dobzhansky. Cultures were raised under constant-temperature conditions at 16°, 21°, or 26° C. In some experiments, specified below, they were raised under naturally fluctuating temperatures. Population density was controlled by using five pairs of parents of specified age limits, left to oviposit 48 hours in a vial before transfer to a fresh vial. Conditions of alternating light (12 hours) and darkness (12 hours), hereafter specified as "LD" conditions, were obtained with fluorescent lamps, controlled by a time switch. Throughout this paper "dawn" is used to designate the dark-light transition in a natural or experimental culture. Some cultures were maintained in constant darkness, hereafter specified as "DD." When the cultures were ready to pupate, a short strip of cylindrical cotton dental plug was introduced into the vial. Virtually all pupation took place on the tip of this plug, which was then removed from the vial and clipped into a canister that held twenty such plugs. The canister was closed with a stoppered funnel of either lucite or aluminum, according to the light conditions to be administered. It is emphasized that in all these experiments the light schedule was rigorously observed. *All* manipulations were performed in absolute darkness when they fell in the dark period. The canisters have several advantages: they permit observation of eclosion time (by inverting and shaking out flies through the funnel) in twenty cultures simultaneously; falling food is avoided; and in a given experiment twenty such canisters may be handled with effective simultaneity, so that the huge number of flies permits a precise estimate of the timed event.

Pattern of Emergence under LD Conditions.—Figure 1 (*A, B,* and *C*) shows the distribution of emergence in LD cultures, maintained at three temperatures and observed hourly. The emergence activity of a culture is quantized: it occurs in bursts in the hours following dawn. It is inhibited throughout those hours of the late morning and afternoon that are in nature the hottest and driest (Fig. 2). There is, then, a 24-hour rhythm of emergence, no matter what the temperature conditions are. The adaptive significance of restricting the allowed period for emergence activity to the coldest and wettest hours of the day is clear enough: emerging flies lose water at a rate at least double that of mature flies and fail to expand their wings properly when the humidity is too low.

The envelope of all the daily spikes forms a distribution, skewed to the right, spreading over several days, the number of which depends on the temperature. The precise shape of each spike is a function of its position in the envelope; the earliest peak is skewed to the left, later peaks to the right (Fig. 3). The general shape of peaks differs, moreover, at the three temperatures; the distributions are in general skewed more to the right at the higher temperatures.

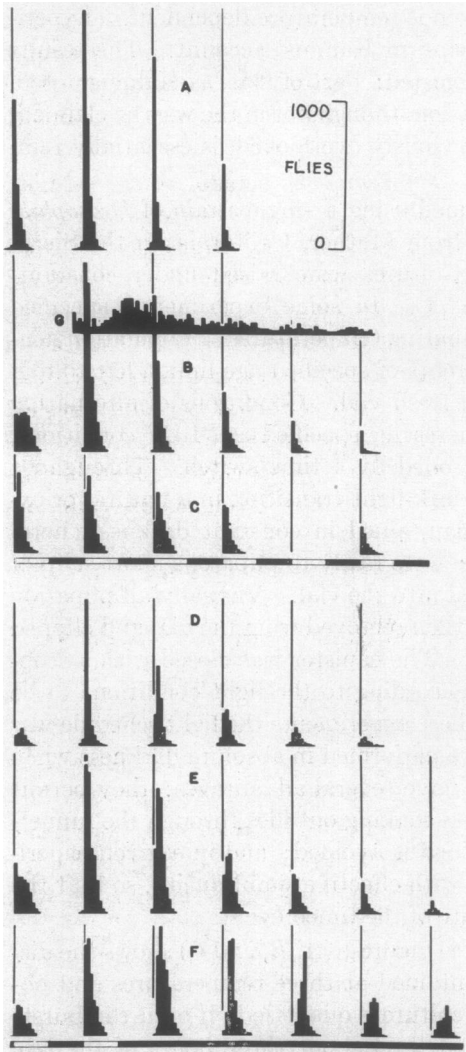Explanation of the changing shape of peaks within the envelope is developed in a

Fig. 1.—The daily rhythm of eclosion. *A* (26° C.; total flies = 7,962), *B* (21° C.; total flies = 9,181), and *C* (16° C.; total flies = 6,209) are from cultures maintained throughout their development under LD conditions, as indicated by the light regime represented under *C*. *D* (26° C.; 4,935 flies), *E* (21° C.; 10,826 flies), and *F* (16° C.; 12,074 flies) are from cultures transferred, without temperature change, from LD to DD, as indicated by the light regime bar under *F*. *G* shows the aperiodic distribution of eclosion that is seen in cultures raised in DD conditions.
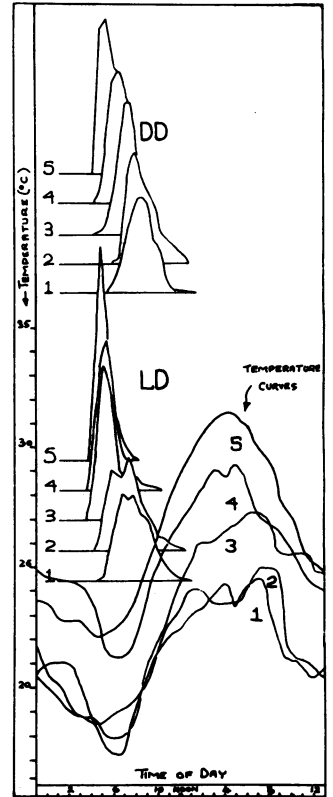

Fig. 2.—The emergence rhythm under natural conditions for five consecutive days. Half the cultures were exposed throughout their life to the daily cycle of light and dark (LD), and half were transferred at the time they pupated to constant darkness (DD) until they eclosed. The number on each temperature curve applies to the 24-hour period the temperature of which significantly affects the correspondingly numbered eclosion peak. As shown in the text, this is the previous 24 hours.

later section.   Here it is noted that this circumstance raises difficulties in selecting a single statistic representative of the time of a given emergence burst.   Means and modes, but especially the former, shift with culture age in a way that is almost certainly not a function of the timing system that we wish to characterize.   This mensuration problem is not pursued further here.   For present purposes the modal hour is used to define the time of the peak.

Demonstration or the Endogenous Clock.—The fact that control of the 24-hour rhythm involves an endogenous clock can be shown by five types of observation: (1) *Persistence in DD:* Figures 1 (*D, E*, and *F*), and 2 show that rhythms in cultures reared in LD under four different temperature regimes persist when the cultures are transferred to DD without changing the temperature regime.   In all four cases, no precisely periodic variable is known to continue in DD in such a way as to act as an exogenous signal for the persisting rhythm.   This observation is not in itself critical. The objection has properly been raised by earlier workers that periodic physical variables like air ionization might persist and be responsible for control of the rhythm.   (2) *Resetting and inversion of the rhythm:*   The plausibility of such criticisms is reduced but not fully eliminated by the fact that the cycle of emergence activity can be completely inverted by detection and experimental use of the physical variable normally acting as reference point for the organism.   In the case of fly emergence this is the dark-to-light transition at dawn:[6] when the length of the light phase in LD conditions is changed to, e.g., 6 hours light (18 hours dark), the position of the emergence peak remains fixed relative to the dark-light transition rather than to the light-dark transition.   In the laboratory emergence can be placed at any clock hour desired simply by subjecting the culture to a single experience of dark-to-light transition.   In Princeton we have had rhythms persisting in DD following the experimental establishment of "dawns" at the following clock hours (EST): 02:00, 06:00, 08:00, 09:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, and 22:00.   Cultures established with dawns on one clock schedule can, moreover, be completely reset to a new schedule by a *single* experience of dawn at a new time (unpublished data).   (3) *Initiation of rhythms in aperiodic cultures:*   Cultures raised from eggs in DD show no emergence rhythm but can be made to develop one by a single light shock,[9, 4, 5] as in Figure 5.   This is perhaps the most remarkable single feature of the entire system.   Larvae, emerging from eggs that have not experienced a 24-hour periodicity of any signal, immediately start measuring off such intervals following experience of a single *unrepeated* event corresponding with the reference point for the cycle in nature.[10]   As in resetting experiments (see 2 above), the experimental reference point can be placed at any clock hour.   (4) *Loss of time under hypoxia:* The clock may be stopped by hypoxia.   A culture was subjected to 15 hours of nitrogen, supplied (with the usual traces of oxygen) from a commercial tank.   The first peak following treatment was delayed 15 hours.   Time was lost corresponding precisely with the time during which metabolism was effectively stopped.   The second peak, however, was delayed only 10
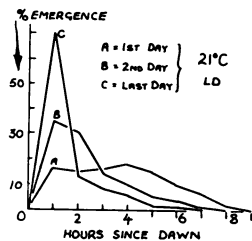

FIG. 3.—Emergence peaks from the first, second, and last day of the culture given in Fig. 1, *B*, to show how the distribution skews progressively to the right with culture age.

hours; and the second, third, and fourth peaks showed an entirely new cycle of 24-hour periodicity, 10 hours out of phase with the first. It will be seen later, after discussion of temperature effects, that the differential response to hypoxia that exists between the first and all subsequent peaks following treatment is highly significant.[11]   (5) *Evidence of a Q_{10} greater than* 1.0: Figure 1 (*D, E,* and *F*) shows that the rhythms initiated at 16°, 21°, and 26° C. under LD conditions show a
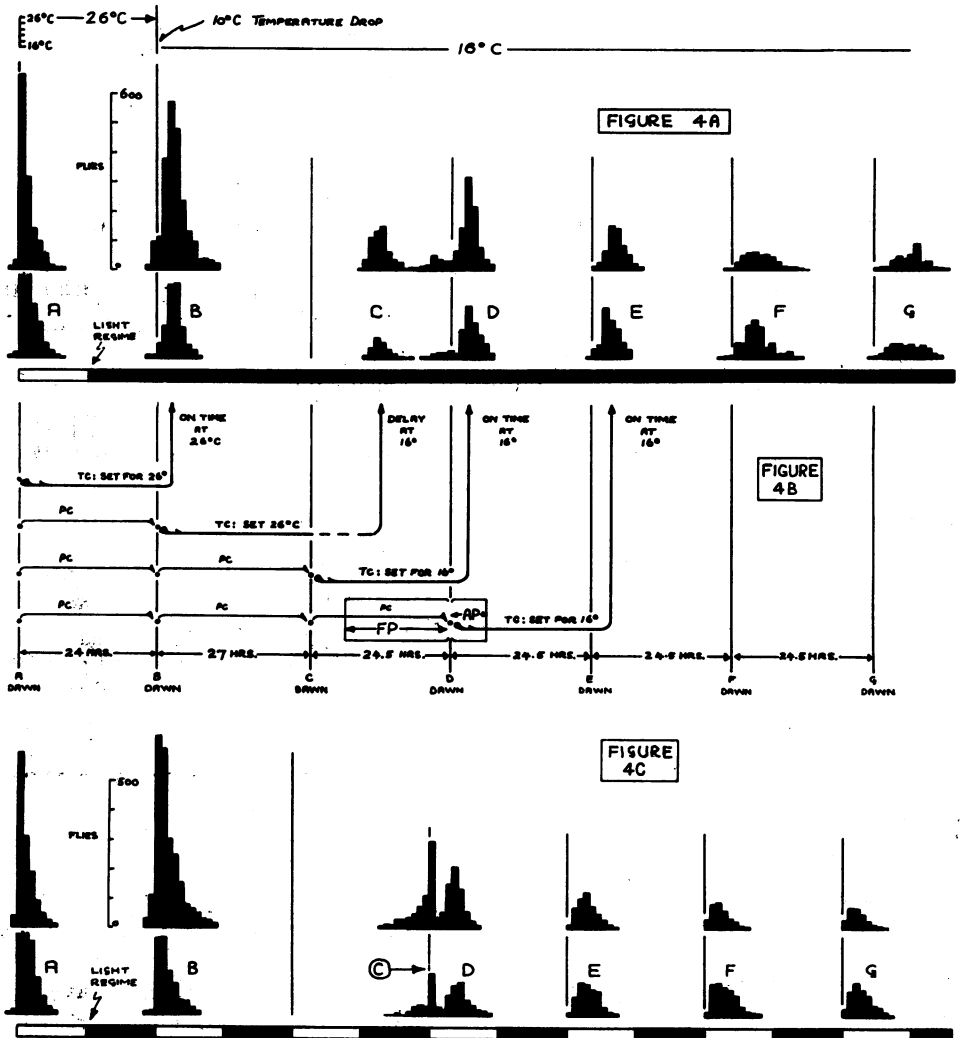

FIG. 4.—See text for explanation. PC = primary clock measurement; TC = terminal clock; AP = allowed period; FP = forbidden period.

very slight though real temperature sensitivity when they are left to persist in DD conditions.[12]   The unmonitored rhythm in DD at 26° C. is 24 hours, and at 16° it is 24.5 hours.   Thus the Q_{10} is about 1.02 over this range.   Were the rhythm monitored by some uncontrolled aspect of the 24-hour cycle of physical change, the Q_{10} would be 1.00.

In view of the above facts, especially the last two, no doubt remains as to the endogenous or metabolic nature of the timing control system.

Synchronization of Emergency by an Enforced Waiting Period.—In all experimental cultures the eggs are laid within a 2-day period, yet emergence activity, commencing about 17 days later, is spread over a period of up to 8 days, depending on various conditions, especially temperature.   It is obvious that all the individual flies in a culture population are by no means synchronized in development throughout their entire history: some develop much slower than others, and yet, when they emerge, the population variance in development time is partitioned into quantized packets 24 hours apart.

It is a matter of definition that the endogenous clock ultimately controls this partitioning of the population into groups of flies effectively synchronized in the time of day chosen for the final act of eclosion.   It is necessary to distinguish, therefore, between synchronization of developmental stages (which clearly does not exist in the culture as a whole) and synchronization of clocks providing information for the control of the final eclosion act.

The precise shape of the daily burst of emergence activity, and especially the way this shape changes with culture age, are striking phenomena shown for the first time in the data given here.   They strongly suggest that synchronization of development is accomplished late in the history of the individual fly by the enforcement daily of a period that is forbidden either for emergency itself or for the initiation of processes leading up to it (see below).   Suppose the 24-hour pe-

FIG. 5.—The induction of 24-hour rhythms by unrepeated stimuli (4 hours of light) applied to cultures raised in constant darkness.   The bars below *C* and *H* indicate the time of the stimulus.   Temperature   conditions   before, during, and after the light treatment are indicated.   *F* shows a rhythm induced in 16° cultures by exposure to 4 hours at 26° C. without light being shown.   *A* is the control culture. The time (EST) of the light shock is different in each experiment.

riod to be partitioned, by means of information from the endogenous clock, into a relatively short allowed period (the data suggest 6 hours or less) and a long (18-hour) forbidden period.   Flies that happen to be ready for emergence within the short allowed period emerge without further delay.   However, flies becoming ready for emergence at random times within the forbidden period are required to wait onset of the next allowed period.   Such a model, entirely formal as it is, explains three major facts: (1) it explains how effective synchronization within daily periods of 6
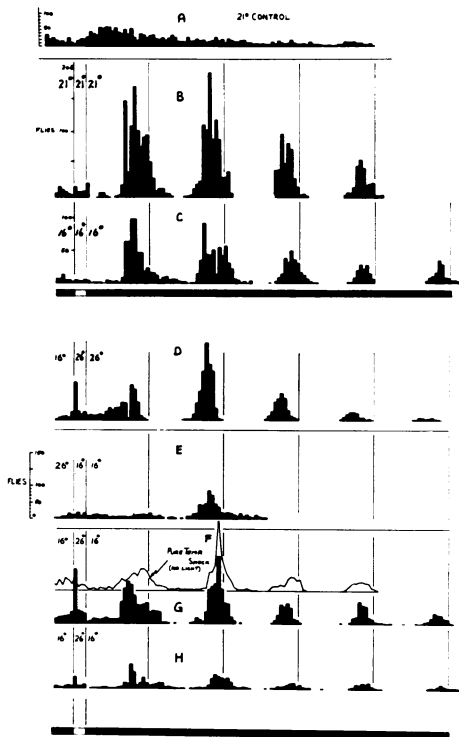
hours is accomplished among individuals whose development rates show in other respects the variance of a nearly normal distribution spread over 8 days; (2) it explains the way the peak is skewed heavily to the right within each allowed period (this is due to the fact that at the beginning of the short allowed period the bulk of the emerging flies have long since been ready and emerge immediately on removal of restraint); and (3), most significantly, the model also predicts the way in which the shape of the daily peak is observed to change with culture age. Each emergence peak consists of two fractions: AP is the fraction composed of flies whose completed development falls by chance within the allowed period; FP is the fraction of flies whose completed development fell within the preceding forbidden period and were required to wait. For each culture there will be only one day when AP/FP is large, viz., the first day of emergence, containing the fastest flies in the culture. In this case the emergence peak should be either normally distributed within the allowed period or even skewed to the left, and this is precisely what is observed (Fig. 3). As the culture becomes older, AP/FP should become progressively smaller and the skew to the right should increase, which, again, is what is observed (Fig. 3).

The utility of this model for explanation of peak shapes leads to its adoption here, but data on temperature effects, to be discussed now, indicate that the restraint exercised in the forbidden period is not on the act of actual eclosion itself but rather on the initiation of special processes involved in the measurement of the last 24 hours prior to eclosion.

Temperature Relations of the Clock System.—When cultures raised in LD conditions at 26°, 21°, and 16° C. are transferred without temperature change to DD conditions, they show persisting rhythms, the periodicity of which is to all intents and purposes 24 hours. The slight Q_{10} for the interval 16°–26° is certainly significant for the elimination of exogenous control, but it is primarily remarkable for its small size. The only certain departure from 24 hours, moreover, is in the 16° cultures, and 16° is the greatest departure from the optimal temperature of the species (about 22° C.). The timing system, to this extent, satisfies the prerequisite for adaptive utility of being nearly independent of temperature; but, as in the bee,[7, 13] the independence has its limits, and the limits are those within which natural selection for it has operated. The half-hour lengthening of the periodicity at 16° C. in DD is ignored, for simplicity of expression, in the remaining discussion, and, when reference is made to temperature independence, it is with the implicit understanding that the Q_{10} for the 16°–26° C. interval is, in fact, about 1.02 rather than 1.00.

There are, however, other temperature effects which at first sight raise severe difficulties for the view of temperature independence, even to the extent and within the limits indicated above. These were first observed by Kalmus, although, as will become evident, his observation did not cover one critical fact. He maintained[4, 5] that, when an LD culture at high temperature (say 26° in our work) was transferred to DD and the temperature was simultaneously dropped (say to 16° C.), then the periodicity lengthened, and, conversely, that transfer from low-temperature LD to high-temperature DD elicited a much-shortened periodicity.

From this position, based, evidently, on observation of only the first peak following the temperature change, Kalmus sought to develop explanations[4, 5] of the way the rhythms persist at 24 hours in DD, whether temperature is high or low,

provided that it is not changed on transfer to DD (Fig. 1, *D, E*, and *F*). And he attempted to develop these explanations on the basis that the processes involved in the clock system were fundamentally temperature dependent, "obeying van't Hoff's rule." His 1940 papers involve two lines of explanation. In one[4] he discusses the phenomena in Semon's terms of biological "memory"; thus the fly learns the interval between dawns as a "subjective time" which is imprinted on a recording system that he compares to a gramophone record, the speed of which is temperature sensitive. When the record is replayed, and thus acts as a clock, at a different temperature without benefit of external signals (dawns), the "subjective time" lengths or shortens according to the temperature change.

Figure 4*A* shows results from two replicate experiments of the type which led Kalmus to the view outlined above. A 26°–16° C. drop accompanies the LD to DD transfer.[14] The replicates indicate the precision of the phenomena concerned. The temperature drop certainly results in a prolonged delay (12 hours in this case) of the first subsequent peak (peak *C* in the figure). But this error does not persist. a fact evidently overlooked by Kalmus: the whole system reverts immediately to a periodicity that is essentially 24 hours and, what is more, a periodicity only 3 hours out of phase with that obtaining before the temperature shock.[15]

Without any external signals, the flies emerging in peaks *D, E, F*, and *G* (Fig. 4*A*) after the temperature shock have good information on the length of a day and fair information on the position of the previously established dawn, in spite of the 10° change that has occurred since the last exogenous information was received on either of these items. Their information is endogenously derived and must be received from a system that is therefore in some sense a temperature-independent clock. It is only the flies emerging in peak *C* whose timing is seriously affected by the precipitous temperature change. In other words, temperature sensitivity is restricted to the terminal measurement of 24 hours immediately prior to eclosion: the flies in peak *C* were the only ones measuring the last 24 hours of pupal life when the temperature drop occurred. All available data, including some to be discussed separately later, demand this strong distinction between the temperature characteristics of the terminal measurement of 24 hours and all those which precede it.

The existence of a temperature-insensitive clock as a piece of inherited physiological machinery, absolutely independent of any ontogenetic learning process, is demanded unequivocally by the experiments in which 24-hour rhythms are elicited at 26°, 21°, or 16° C. by *single* light or temperature shocks imposed on cultures raised in DD conditions and completely aperiodic prior to the single stimulus. These experiments (Fig. 5) have already been noted.[16] Kalmus reported the induction of rhythms by single stimuli and developed a second line of explanation for their interpretation. Again viewing the clock system as fundamentally temperature dependent, he supposed[5] that quasi-independence (exemplified by the present Fig. 1, *D, E*, and *F*) was achieved by operation of temperature regulators set at dawn. These regulators, he maintained, could only be set during the illuminated period immediately following dawn (i.e., their "setting" was light dependent), and their adjustment was made in anticipation of temperature conditions throughout the ensuing 24 hours corresponding with those perceived at the dawn period. He did not clarify how, in nature, the fly related temperature perceived at dawn to the shape and height of the temperature curve in the ensuing 24 hours.

It is unnecessary to dwell on the formal inadequacy of Kalmus' scheme, since the facts do not fit it. It will be observed from Figure 5 that no matter what the temperature is (16°, 21°, or 26°) before, during, or after the light stimulus, the periodicity elicited is 24 hours (cf. Kalmus, *Nature*, **145**, 72, 1940).

In brief, then, the experiments with single stimulations (Fig. 5) eliminate not only any form of an ontogenetic memory hypothesis but also any other (such as Kalmus' second line of explanation) which avoids invoking some form of temperature-independent time measurement on the part of the fly.

A New Model for the Clock System in Drosophila.—The crux of the whole situation is the distinction, already made here for the first time, between the terminal measurement of 24 hours, immediately prior to eclosion, and all those that precede it in the earlier history of the fly. Kalmus' important discovery of extreme temperature sensitivity in the system applies only to the terminal measurement, as shown by Fig. 4A.

The terminal measurement is unique not only in its temperature dependence but also in its differential oxygen sensitivity, and the conclusion is at the very least suggested that it involves physiological processes distinct from those involved in the earlier measurements.[17] It is therefore convenient to speak of a "terminal clock" comprising temperature-sensitive events occupying (when properly "adjusted") the 24 hours immediately prior to eclosion, and to distinguish this from a "primary clock" (temperature insensitive within ecological limits) that measures throughout the development of the fly 24-hour intervals between reference points in phase with the last-seen dawn.

No attempt will be made here to develop a complete scheme for the entire system. The purpose of this paper has been to present those facts that indicate the existence of a temperature-independent component in the timing system, and it only remains to indicate present understanding of the way the two clocks, defined above, are related in ultimate control of emergence time.

Previous discussion of the existence of allowed and forbidden periods within the 24-hour cycle was entirely formal and left open the question as to precisely what event it is that suffers an enforced wait during the forbidden period. The simplest hypothesis is that it is the brief event (minutes) of eclosion itself. This is unacceptable for two reasons: first, it leaves unexplained the major fact of temperature sensitivity in the terminal measurement; second, it would follow from this hypothesis that flies completing development at random times throughout the forbidden period would not achieve synchronization until the very last moment coincident with the removal of restraint at the onset of the allowed period of the very day on which they emerge. This is not the case. Flies whose eclosion is advanced or delayed (Fig. 4A) by a precipitous temperature change are nevertheless synchronized when they do eclose, even though this is not coincident with the onset of an allowed period defined by the primary clock. It follows, therefore, that the synchronization of final development is achieved before the ultimate act of eclosion itself.

The distinctness of the terminal clock, which is assumed to be a unique event, invites the hypothesis that it is initiation of this process that is synchronized by an enforced wait. On this view, all the flies in a culture are synchronized in their earlier history only with respect to information from their primary clock and are not synchronized in their developmental stages until the point is reached at which they

are ready for the processes involved in the terminal clock measurement (TC). Their development finally becomes synchronized only in the last 24 hours, since to initiate TC they must await onset of an allowed period defined by primary clocks previously synchronized by exposure to a common dawn.

This model is represented schematically in Figure 4B, which interprets clock control of the peaks in Figure 4A. Consider the history of flies in peak E: having once seen dawn as a reference point (dawn A in Fig. 4A), the primary clock measures intervals that are very nearly 24 hours[15] throughout the remaining days of development, in spite of the temperature change, and defines the forbidden period between dawns C and D during which flies otherwise ready are prevented from initiation of TC which takes place at dawn D. This terminal measurement, although temperature sensitive, is, in fact, adjusted to take approximately 24 hours for completion at dawn E, given 16° C., which was the temperature obtaining during the previous 24 hours. The same account applies for peak C, but in this case temperature adjustment on the terminal process was made in anticipation of the prior conditions of 26° C. The fact that the temperature was then dropped to 16° accounts for the 12-hour delay of the terminal measurement controlling peak C.

Regulation of the Temperature-sensitive Terminal Measurement.—This discussion began by noting that near-independence of temperature must surely be a prerequisite for a useful organic clock. Re-examination of the situation in *Drosophila* reveals the anticipated temperature-independent clock but finishes with the perplexing conclusion that the all-important terminal measurement of 24 hours is timed by a process that is conspicuously temperature sensitive. This situation is not quite so paradoxical as it may seem, for the two following reasons.

1. The terminal measurement, although certainly temperature sensitive, is subject to regulation of some kind. Discussion, even formal, of how it is regulated would require more data than can be handled here. Evidence on hand conforms with the view that the terminal measurement is initially adjusted in anticipation of an area under the ensuing 24-hour temperature curve comparable with that experienced during the previous 24 hours. As the weatherman knows, this is a reasonable gamble. And in noting that such a system still permits "errors" such as peak C in Figure 4A (which falls in the normally hot, dry portion of the day), there is an important point to bear in mind. The fly has evolved in relation to conditions that never confront it with consecutive days whose temperature regimes are the equivalent of 24 hours at 26° C. and 24 hours at 16° C.

It is necessary to emphasize only one further point in connection with adjustment of the terminal measurement. No matter how the regulation is effected in a concrete physiological sense, it is a logical necessity that the fly must possess, as part of its regulatory equipment, the equivalent of a temperature-insensitive timepiece to act as reference in effecting the adjustments manifested by peaks D, E, F, and G in Figure 4A.

2. In nature light is presumably seen at each dawn, and under these conditions even the "unnatural" precipitous temperature drops employed in our experiments do not have the inadaptive consequence of placing emergence in the usually hot, dry second half of the day. Figure 4C suggests that, when light is seen at dawn, the allowed period for emergence is more rigorously defined than when it is entrusted to definition by the endogenous clocks alone, and it shows that delayed

peaks falling within the later part of the day are reinhibited and required to await the following dawn.

[1] G. Kramer, *Ibis*, **94**, 265, 1952.

[2] L. Pardi, and F. Papi, *Z. vergleich. Physiol.*, **35**, 459 and 490, 1953.

[3] H. Kalmus, *Biologia generalis*, 11, 93, 1935.

[4] H. Kalmus, *Acta med. Scand.* (suppl.), **108**, 227, 1940.

[5] H. Kalmus, *Nature*, **145**, 72, 1940.

[6] E. Bünning, *Ber. Deut. botan. Ges.*, **53**, 594, 1935.

[7] O. Wahl, *Z. vergleich. Physiol.*, **16**, 529, 1932.

[8] F. A. Brown, Jr., and H. M. Webb, *Physiol. Zoöl.*, **21**, 371, 1948.

[9] Light is not the only factor which can set the clocks in DD cultures. 5° and 10° temperature shocks can also do this (Fig. 5) (cf. Kalmus, *Nature*, **145**, 72, 1940, to the contrary). But it appears that temperature cannot reset clocks once established in an LD regime. The facts here are complex and will be discussed elsewhere.

[10] In the case of DD cultures which shift from aperiodic to periodic activity, following single stimuli, the question arises whether these single stimuli initiate clocks in all individuals or simply reset clocks, formerly out of phase, into a condition of synchrony. These alternatives cannot be resolved with present techniques in *Drosophila*, where the periodicity can be measured as a function only of the population and not of the individual. We are inclined, however, to favor the view that aperiodic cultures represent populations of individuals whose clocks are already running and are merely out of phase. Cases in the literature where periodism in DD is eventually lost all involve populations; the longest-enduring periodicities in DD that have been published (F. E. Lutz, *Am. Museum Novitates*, No. 550, 1932, and O. Park, *Ecology*, **16**, 152, 1935) are based on the study of individuals.

[11] Kalmus (*Biologia generalis*, 11, 93, 1935) first performed an experiment of this type. He reported delay of only the first peak following the treatment. In view of the model presented in this paper, it is clear that critical demonstration of stopping the principal 24-hour measuring device depends on the delay, shown here, of the second and subsequent peaks.

[12] It will be noted from Fig. 1 (*D*, *E*, and *F*) that on the first day of DD condition (following transfer from LD) the peak is slightly delayed. Evidently the normal onset of light in the morning acts as a supplementary stimulus to eclosion in those flies that are ready. This boosting action of light is also very clear in Fig. 5, where the 4-hour light stimulus applied to DD cultures has two effects: (1) it synchronizes the clocks in flies still a long way from eclosion; but (2) it also promotes an immediate burst of eclosion in flies almost ready. Notice also how, in Fig. 4*C*, the light at dawn *D* spectacularly stimulates the flies belonging to the reinhibited peak *C*.

[13] H. Kalmus, *Z. vergleich. Physiol.*, **20**, 405, 1933–1934.

[14] The reciprocal experiment, viz., a 16°–26° C. temperature rise accompanying LD to DD transfer (as well as many other different temperature shifts) have been carried out in this study. The 16°–26° change elicits an advance of only the first peak following treatment; as in the 26°–16° experiment, all subsequent peaks fall into a normal 24-hour periodicity in phase with the original. For brevity, the rest of this paper is based only on the experiment involving the 26°–16° drop, but all arguments based on it are fully supported by the other experiments. Indeed, it is noteworthy that experiments involving a temperature rise, as from 16° to 26°, show even less of a disturbance on both primary and terminal clocks (see later discussion in text) than do those involving the 26°–16° drop. This asymmetry of the temperature effect is curious and unexplained. Experiments involving temperature drops rather than rises are useful simply because the exaggeration of the temperature effect facilitates its detection.

[15] The single measurement by the primary clock of the period between dawns B and C (Figs. 4A and 4B) was, by inference from the position of peak D, 3 hours slow. Peak D can be displaced by light shocks given in the neighborhood of dawn C. This technique has permitted verification of the fact that the primary clock's reference point corresponding with dawn C is, in fact, 3 hours late: light shocks given at this hour do not displace peak D, but, if given 1, 2, or 3 hours later, they displace the peak correspondingly 1, 2, or 3 hours to the right. Other evidence indicates that this temperature sensitivity of the primary clock is restricted to virtually instantaneous changes greater than 5° C. and is considerably greater near the reference point (dawn equivalent) than elsewhere in the cycle.

[16] It is remarkable that the first peak in these experiments comes about 18 hours after the light shock. The significance of this is not clear, and the scheme presented later in this paper does not explain it. Several features of our data suggest the fractionation of the 24-hour cycle into 6- and 18-hour components.

[17] The possibility that the terminal measurement is a function of the same clock that measures the earlier 24-hour periods has, of course, been given careful consideration. An attempt has been made to explain the characteristic temperature sensitivity of the terminal measurement in terms of the well-known physiological effect of temperature overshoots and undershoots (cf. F. J. Ryan, *J. Exptl. Zoöl.*, **88**, 24, 1941). This approach has been rejected, since it does not explain several features of the experiments shown in Fig. 4 and others to be discussed at a later time, when the possibility will be given more explicit treatment.