Exogenous and Endogenous Components in Circadian Rhythms

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PREFACE

The main topic of the symposium "Biological Clocks" says nothing about the types of clocks we will be concerned with. It is an open question whether the clocks run continuously or stop after one revolution and have to be started anew. Also the term "clock" does not imply that one revolution is finished in 24 hours. Watches are instruments to measure time. Organisms have to measure time-spans of quite different lengths and for different purposes. Each measurement of speed, for instance, needs timing and mostly within the limits of milliseconds. One can expect, therefore, that organisms possess several clocks with perhaps extremely different periods [1]. There is no need for these clocks to run continuously; for some purposes it would be sufficient if the clock were started always at the beginning of timing (principle of a sandglass, stop watch). "Biological clocks" therefore is a more general concept than what will be mainly discussed at the symposium. On the other hand, the term "circadian," as introduced by Halberg [2] and used in 45 percent of all titles concerning the special clock-problem, comprises two different things: a) the average period of the clock is about 24 hours; b) the clock runs continuously.

At present the circadian clock can be studied only by measuring the periodic course of one or more functions in an organism. Still, nobody can say how these observable functions are related to the clock, how well respectively "the clock" is represented by one function. To answer this question, it may be in the future still more necessary than now to measure several functions simultaneously in one organism, and to observe how they are synchronized or become desynchronized during certain experimental conditions. Nevertheless, many circadian problems can be solved if only one function is followed. It may be that to answer some special questions one or the other function is more useful. But with respect to the general mechanism of the clock, all functions are nearly equivalent. Scientists who work on eosinophiles or on locomotor activity are surely not nearer to, but may be also not so much further from, the clock than those who train animals to feed at definite times of day or to run always toward the same quarter. It will be more important—at least with regard to the problem of synchronization—if we can demonstrate that the results of certain circadian experiments are always equal, independent from whatever functions may have been selected (compare Figs. 17 and 18).

An organism, executing under natural conditions a day-night periodicity, does not necessarily possess a circadian one. The periodicity can be purely exogenous. In that case, environment is the real and only cause of the rhythm which ceases in artificial constant conditions. Contrary to this, the circadian rhythms are endogenous, that means: they are caused in the organism itself. The periodic environment operates only as a synchronizing agent. Periodic factors of the environment, which are able to synchronize a circadian periodicity, have been designed as Zeitgeber [3, 4] or synchronizer [5]. Entraining agent, time-giver, time-cue, and others are used as analogues. As it is not yet decided what term may in general be accepted by the English literature, the German "Zeitgeber" will be used here throughout. The question, whether a biological periodicity is endogenous (circadian), and whether an environmental factor is a Zeitgeber, has to be tested in each single case by certain experiments. Some experiments of this type will be discussed here. So far as they have been carried out in our own laboratory, they are to a great extent a consequence of the most stimulating visit Pittendrigh paid last year to Germany. The results have been obtained by collaboration with Iwan Diehl, Ursula Gerecke, and Rütger Wever.

I. ENDOGENOUS COMPONENTS

I.1. The Problem of Endogenous and Exogenous

To establish a periodicity as an endogenous one, it is necessary to exclude all possible Zeitgeber. Therefore, the organism to be tested must be transferred to what is called "constant conditions." But a priori it is not clear what sort of environmental factors should be considered as potential Zeitgeber. In keeping constant all variables of the environment most commonly controlled, as light, temperature, and so on, three results of such an experiment are possible:

a) The periodicity ceases suddenly or damps out within few periods. This result is neither a convincing proof against "endogenous" nor for "exogenous." It could be the case that a periodicity,

although endogenous, becomes unobservable by the tested function because the environmental factors chosen for constancy are too unfavorable (environment too hot, too cool, too bright, too dark, and so on; compare chapter II.3).

b) The periodicity continues and that with a period of exactly 24 hours. In this case, one can not exclude that an overlooked or unknown periodic factor of the environment was effective as Zeitgeber. All experiments in constant conditions, the results of which show an unaltered frequency and phase of the organism, do not prove an endogenous periodicity and still less that this periodicity was inherited.

c) The periodicity continues, but with a frequency deviating by a certain, more or less constant, amount from that of earth-rotation. If there is no other periodicity in the environment (perhaps a tidal one), with which the organism is in synchrony, then the periodicity is really endogenous.

This spontaneous frequency or, to use Pittendrigh's phrase [6, 7], the free running period, is the only convincing evidence of an endogenous (circadian) periodicity. Thereby, one has to mention that a frequency may not be ascertained without measuring several periods. Also, in studying the circadian clock, at least 5 to 7 periods should be registrated before speaking of a spontaneous frequency.

The free running period we can observe in an organism is, of course, nothing like a physical constant. Organisms as open systems are always correlated to the environment. Behavior and function are results of an "inside-outside" coaction. Therefore, we can not expect that the spontaneous frequency of an organism will be the same in all conditions. In a constant environment the period may depend as well on the functional state of the organism—breeding time or anoestrus for instance—as on the special environmental conditions, as intensity of illumination, temperature, and so on. That the organism, although living in a constant environment, behaves rhythmically, we call "spontaneous" and "endogenous." The actual value, however, of the rhythm, the frequency, is determined by all circumstantial conditions—external as well as internal. These statements have already been made by Pfeffer [8].

The term "spontaneous frequency" means that the periodicity arises in the organism spontaneously without external periodic stimuli. Thus "spontaneous" applies to neurophysiological usage. Spontaneous rhythm in neurophysiology means: rhythmic output from the living system in contrast to continuous constant input from the environment. (Without input—no Life!) Heart beat and the rhythmic impulses from the respiratory center are spontaneous. Both centers operate rhythmically even if the cellular milieu is kept constant. The frequency of the impulses depends on all conditioning circumstances, e.g., on constant temperature or on constant CO_2-tension. It is well known that the sensitivity, or better responsiveness, of such a system to an irritation changes periodically. To describe such a rhythm in terms of "phase-shift," of "autophasing," or of "continuously resetting by CO_2-shocks" would be quite unusual.

Discussing the problem of "spontaneous," a few words seem to be necessary with regard to a hypothesis suggested by F. A. Brown. Even in 1957 he emphasized, "that organisms in constant conditions may retain unaltered phase relationships with the external physical cycles even for periods of month," and, "that the clock system maintains its regular frequencies through some kind of an external pacemaking signal which continues to be effective under what is usually deemed 'constant conditions' " [9]. Confronted with such results, everybody would agree that Zeitgeber must have been operating in these experiments, although they are not necessarily "still unknown external factors" [10]. During the last two years, Brown has put into his hypothesis the free running period deviating from 24 hours [11]. He describes the spontaneous frequency as an effect of "autophasing," whereby it is not always clear whether this autophasing is the result of external time-cues or of a constant environment [12]. Both explanations presume a periodic changing sensitivity of the organism. If time-cues were operating, as a consequence of the varying sensitivity, the organism should be in synchrony with the cues; deviating periods are out of the range of entrainment, and the time-cues behave in these cases as a constant environment with respect to the organism. If, on the other hand, there are no time-cues, but real constant conditions, then the coaction of a rhythm (with a changing sensitivity) with such a constant environment is again in correspondence with the definition of "spontaneous" given above. One last example: The frequency of an electronic circuit depends on inductance and capacity. The system oscillates continuously and spontaneously if the inevitable losses of energy are replenished by an anode-voltage via feed-back. If one of the parameters of the circuit is made sensitive to light by use of a photocell, the frequency of the system will become a function of the intensity of illumination. Nevertheless, the oscillation remains spontaneous (endogenous); only the actual value of the frequency is determined by internal as well as by external factors. It is evident that such a system reaches once during each period a point of highest sensitivity against light. It would be unusual, however, to describe the oscillation as an effect of "autophasing," as "resetting the phase of the oscillation," or as an "exogenous" periodicity. The consequence would be that one frequency of a system would be called "frequency" and another one "resetting."

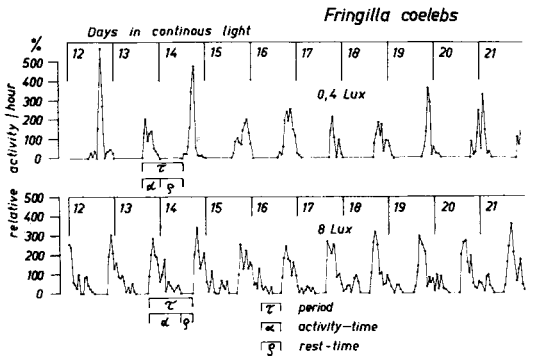

FIGURE 1. Activity of a male chaffinch. Constant illumination of two intensities, 0.4 lux (above) and 8 lux (below). Ordinate: Continuously registrated activity per hour.

I.2. The Spontaneous Frequency

As a simple example of what is called a spontaneous frequency, Fig. 1 shows the activity of a caged chaffinch in constant conditions—constant temperature, continuous feeding, isolation from noises, and continuous illumination with an intensity of 0.4 lux (above) and 8 lux (below). The diagram explains two things: a) With an intensity of 0.4 lux the period is longer than with 8 lux; b) Within one period two fractions can clearly be divided: activity-time, when the bird is awake and jumps around in its cage, and rest-time, when the bird sleeps [13]. Offering an intensity of 0.4 lux, activity-time is shorter and rest-time longer than offering 8 lux. Both the fractions of the period will be discussed later on. First frequency requires the main interest. The length of each period is easiest to derive from the sharp onsets of activity. But also peaks of activity or the ends of activity-time could be used as reference points between which the period is measured. Without a special examination one can not predict which point of the biological period may be more important or useful in studying the clock. It has been shown, however, in the results of several experiments that, in measuring activity, the onsets scatter less around the average period than other points (compare Fig. 2) [14]. In the following discussion often the onsets, but also sometimes the peaks of activity, will be used for reference.

The accuracy with which an organism keeps its frequency is easy to demonstrate by marking the successive daily delays or advances of onsets with respect to local time in a diagram. In Fig. 2 the sequence of days as the independent variable is drawn on the abscissa. The ordinate represents hours of onsets and ends of activity of a chaffinch, given in Central European time for each single day. Under the conditions of alternating 12 hours of light and 12 hours of darkness (*LD* 12:12), the activity-time of the bird is strongly fixed to the light-time (= time of illumination) [15]. In continuous illumination with an intensity of 0.4 lux, activity starts and ends each day a little later. After 30 days of continuous illumination, the bird becomes newly synchronized with *LD* (12:12) during 12 days and then again exposed to continuous illumination, but now with an intensity of 120 lux. In this high intensity of *LL*, activity starts each day a little earlier. The diagram shows: a) The onsets of activity offer a more precise measurement of period and thus also of frequency than the ends of activity; b) By a continuous illumination with an intensity of 0.4 lux, activity-time is shorter than in *LL* with 8 lux; c) A strong artificial, nearly unnatural Zeitgeber (compare Fig. 14) with alternating 12 hours of bright light and 12 hours of total darkness, catches the free running frequency suddenly; also, after continuous illumination is reestablished, the spontaneous frequency starts at once.

A certain intensity of constant illumination given, the spontaneous frequency varies with each organism tested, and also in one organism the frequency can change without obvious causes. The onsets of activity of 4 chaffinches in *LL* are drawn in Fig. 3. They have been measured in 4 different intensities of illumination during at least 20 days. Although the frequencies vary inter- and intraindividually, each intensity of illumination is characterized by a certain frequency, averaged on all 4 birds. This average spontaneous frequency is lowest in 0.4 lux and highest in 120 lux.

I.3. Frequency, Depending on Light Intensity

Since long ago it has been known that the spontaneous frequency depends on the intensity of illumination [16, 3]. This fact has been confirmed recently in several organisms. In general, the frequency varies in a linear scale with the logarithm of light intensity. A survey on all results so far published is presented in

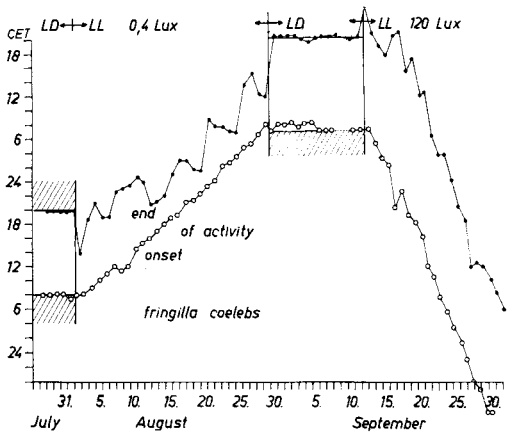

FIGURE 2. Onset and end of activity of a chaffinch in alternating 12 hours of light and 12 hours of darkness (*LD*, 12:12) or in continuous illumination (*LL*) of two intensities (0.4 lux and 120 lux). Ordinate: Daily onset and end of activity at Central European time.

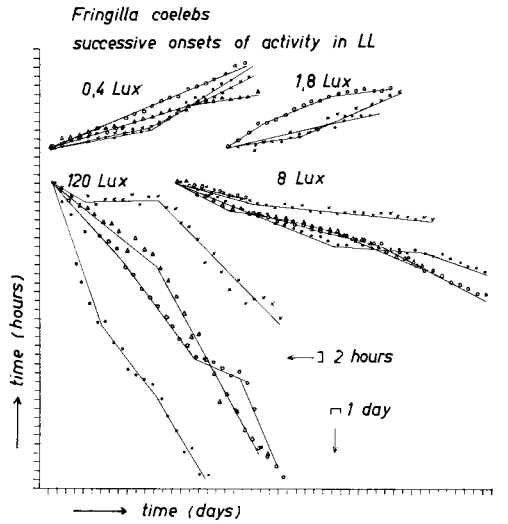

FIGURE 3. Consecutive onsets of activity in 4 chaffinches during at least 20 days. Continuous illumination of 4 different intensities. Delay of onsets with 0.4 and 1.8 lux, advance of onsets with 8 and 120 lux. Notice different time-scale on ordinate and abscissa.

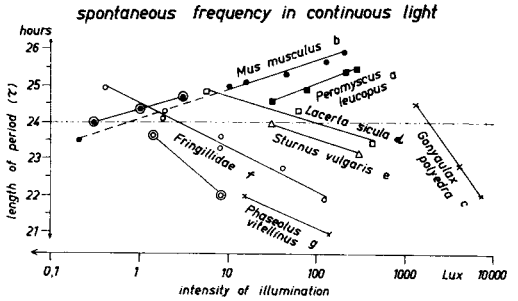

FIGURE 4. Spontaneous (circadian) period of divers organisms in constant environment, depending on intensity of illumination. Each point represents the average of several individuals and periods. *a*) Activity, Johnson [16]; *b*) Activity, ● old and ◉ new experiments; *c*) Luminescence, Hastings and Sweeney [17]; *d*) Activity, Hoffmann [18]; *e*) Activity; *f*) Activity, ○ first and ◎ second series of experiments; *g*) Leaf movements, Pfeffer [19.]

Fig. 4. Each point of the diagram represents the average of several organisms (with the exception of *Phaseolus*), which in each intensity of illumination have been measured during several periods. The figures concerning starlings, finches (1 and 2 series), and mice (2 series) have not yet been published in detail. The results of the first and the second experimental series on mice are close to one another. Contrary to this, finches perform a much higher frequency in the second experiment than in the first. Such a variation of sensitivity to light is by no means astonishing; for instance, it could be expected especially as a consequence of seasonal changes in the functional state of the organism. That the straight line representing a species in Fig. 4, should always have the same slope and position in the diagram by repeated experiments seems to be unlikely. Each intervening treatment of an organism (of whatever kind) may change the spontaneous frequency from one test-experiment to the next. Also quite clearly an organism in *LL* will have different frequencies in an empty cage and in a cage wherein is offered a dark corner to move in. The figures concerning *Phaseolus* are correctly arranged in their relative positions; the absolute value of light intensity used by Pfeffer could only be guessed.

The results of divers authors as they are collected in Fig. 4 suggest the hypothesis that in *LL* with increasing intensity of illumination, light-active animals (finches, starlings, lizards) increase their spontaneous frequency, while dark-active animals (housemice, white-footed mice) decrease it. This rule, when mentioned first, was formulated unsatisfactorily [20, 21]. Discussion with Rawson and his students at Madison contributed to the final version [22]. Until now no results seem to be published which contradict the rule in principle; nevertheless, exceptions may be expected. Details of the rule will be discussed in chapter I.5.

The frequency of an oscillating system is measured as the reciprocal of period. Beside this, the system has within one single period what may be called a speed. This speed can be described in terms of an angular velocity of a rotating vector. Only a true sinusoidal oscillation has a constant speed throughout the whole cycle. In all other oscillations the speed varies within one period. That means: in a circadian oscillation there are fractions of the period during which the system runs slower than in the average and other fractions during which it runs faster. It seems reasonable to assume that in *LD* this varying speed is co-ordinated with the Zeitgeber in some way. Accepting this, the data presented in Fig. 4 suggest that in light active animals increasing intensity of light (or the change from *D* to *L*) tends to accelerate the speed of the system and that decreasing intensity (the change from *L* to *D*) tends to slow it down. The rate of change in speed depends, of course, on the phase when the organism is exposed to higher or lower light-intensities. Moreover, if one looks at an animal in constant conditions, one could expect that the varying speed of the system is correlated to the two fractions of the period: activity-time and rest-time. If one assumes that activity-time coincides with a speed higher than in the average and rest-time with a lower one, a testable prediction can be made: In lengthening the activity-time—i.e., the time during which the system runs faster than in the average—the period must become shorter. This has been shown in many cases. Differences in the speed of the clock have still not been measured directly. But there are two observations which perhaps can be taken as indirect evidence. First, in the hierarchy of rhythms [23, 24] the 2-hour rhythms of activity ("bursts" of activity) are most striking. It has been shown that these

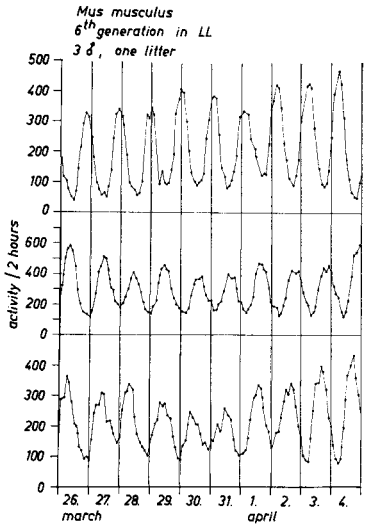

FIGURE 5. Activity of three mice in *LL*. One litter, born November 11th, of the 6th generation, raised in continuous illumination. Original figures smoothed, using a running average of 5 points (compare Fig. 6, left column, F_5).

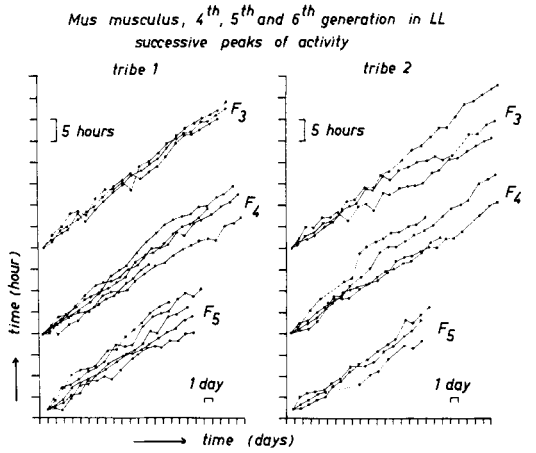

FIGURE 6. Daily delay of the peaks of activity in 6 litters of mice in *LL*. F_3-, F_4-, and F_5-generation of two strains, raised in continuous illumination of about 100 lux.

bursts have a higher frequency during activity-time (in *LL* as well as in *LD*) than during rest-time [13]. Second, animals using the sun as a compass for orientation have to compute the sun-movement with an average angular velocity of 15° per hour. Unpublished experiments indicate that some light-active fish compute more than 15° per hour during light-time and less during dark-time [25]. The question, whether these observations corroborate the hypothesis of an interaction between the Zeitgeber and a varying speed in the oscillating system or not, can not be discussed here in detail.

I.4. Inheritance of Periodicity

If an organism has been shown to possess an endogenous (circadian) periodicity, the question arises whether the periodicity was acquired by the individual during ontogeny or has become part of hereditary disposition during phylogeny. It is self-evident that only the potentiality to behave rhythmically can be inherited. The realization of such a potentiality depends, as in all other functions, on several circumstantial conditions. There is no need for an inherited periodicity to be in full function directly after birth. If the periodicity unfolds only in the growing organism step by step (as in man), this does not necessarily mean that the periodicity has been impressed upon the organism by the environment. All organs or centers of co-ordination necessary for the periodicity have to be fully grown up before an overt periodicity can operate even if it is inherited [26, 27].

Animals which are raised in constant conditions from very early states of development and which, nevertheless, show a spontaneous frequency can be said to have an innate periodicity. Successful experiments of such kind have been carried out in chickens [26] and lizards [28] by raising them from the egg on in continuous illumination or darkness. Also, lizards which grew up in an artificial 18-hour or 36-hour day showed, when tested in constant darkness, natural periods similar to those of normally growing animals and independent from the foregoing lighting regimen [29]. Experiments in which the animals are raised in constant conditions for several generations give no proof for an innate periodicity as long as the period remains 24 hours; the possible effect of a Zeitgeber can not be excluded. If, on the other hand, mammals raised from birth on in *LL* or *DD* develop a spontaneous frequency, one can object that the nursing mothers may have impressed their rhythm upon the young [30]. It may be, however, interesting enough to note the fact that 6 generations of mice raised in *LL* kept all the same spontaneous frequency. The activity of three litter-mates of such a F_5-generation is shown in Fig. 5. The spontaneous frequencies of the 4th, 5th, and 6th *LL*-generation in two strains are in good accordance with each other; this is demonstrated in Fig. 6. Here the successive peaks of activity are marked for all individuals of six litters during at least 20 days. All periods are within the limits of 25.0 and 25.6 hours.

I.5. Three Important Parameters

In constant conditions, spontaneous frequency and activity-time are two parameters of the oscillation easy to measure. A third one, more difficult to ascertain but perhaps equally important, is given by the level around which the system oscillates. The level is not identical with the amplitude. An electronic circuit can oscillate with exactly the same amplitude around

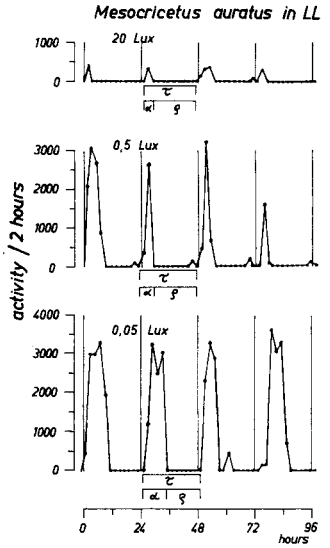

FIGURE 7. Activity of a hamster, measured in a running wheel in *LL* with three intensities of illumination. Increasing rest-time (ρ), decreasing activity-time (α), and amount of total activity with increasing light intensity. Nearly no change of period (τ).

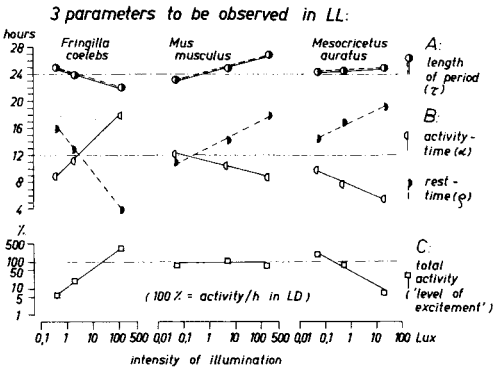

FIGURE 8. Three circadian parameters, depending on intensity of illumination in constant environmental conditions. *A*) Length of spontaneous period; *B*) Ratio of activity-time to rest-time; *C*) Total amount of activity per unit-time (100% = average activity per hour in *LD*). Each point represents the average of 3 (finch and mouse) or 2 (hamster) individuals, measured during at least 6 periods each.

a low or a high level. The two states differ in the amount of energy they spend per unit time. Applying this to biology, one can call the average level around which the system oscillates "level of excitement." Body temperature can oscillate around 37° as an average as well as around 38° with exactly the same amplitude. In this case the average temperature would be the level. The total amount of activity performed by the animal per unit time may be (although imperfect) a measure of this level. Likewise, or even better, one can probably use the metabolism of energy (oxygen consumption) as a measurement for the level of excitement. Evidently,

TABLE 1. PERIOD (τ), ACTIVITY-TIME (α) AND TOTAL ACTIVITY (A) IN STARLINGS DURING CONSTANT ILLUMINATION OF THREE INTENSITIES

LL, Lux	τ, hours	α, hours	A, counts/30 min
0.6	24.3	9.3	117
7.5	23.5	14.7	300
65.0	22.5	16.5	750

neither activity nor oxygen-consumption is a precise measurement of "the level." There are many cases in which the same oxygen consumption coincides with different frequencies. The effective power may play a role. On the other hand, it is clear that the level is an important parameter in each oscillating system. Although there is no clear answer at this time, it seems reasonable not to exclude the question: What does "level" mean in the circadian oscillation of an organism? The terms "light-active" and "dark-active" are meaningful only in the way that light increases activity (or the level) in light-active animals and decreases it in dark-active animals. Without such a causal connection between intensity of illumination and level of excitement (or activity) there would be no separation between light- and dark-active animals. One of the primary reactions of the organism to light is that the level of excitement increases or decreases.

In many of the animals so far studied, level of excitement, spontaneous frequency, and $\alpha:\rho$-ratio (activity-time:rest-time) seem to be correlated. Comparing the three parameters in a dark-active animal in *LL* with three intensities of illumination (Fig. 7), one notices: Higher intensities of light are combined with a smaller amount of total activity, with a shorter activity-time, and with a (only little) longer period. The reverse applies to light-active animals. Indeed, all three parameters are not always equally dependent on the intensity of illumination. It may be that in one organism total activity (= level), in another one period remains nearly constant. In each case, however, at least two parameters seem to follow the rule which is demonstrated in Fig. 8 with respect to one light-active and two dark-active species. Each point of the diagram is the average of measurements on three animals (two hamsters only) during at least 7 days. Quite the same results as shown in Fig. 8 with finches Hoffmann [18] could obtain in experiments on starlings (Table 1). Concerning Fig. 8, a few more remarks may be useful. In finches and mice, the $\alpha:\rho$-ratio is 1:1 when the period is 24 hours. Perhaps from these facts one can derive that also in hamsters the period will be shorter than 24 hours if in very low intensities of illumination the $\alpha:\rho$-ratio becomes 1:1. Further it is remarkable that in mice there is no change in the total amount of activity. This coincides with the observation that mice

easily alternate between light- and dark-activity and that they always have two peaks of activity, the main one during dark-time and the second one during light-time. Other conclusions will be referred to later (compare Fig. 15).

The results as presented in Fig. 8 and in Table 1 encourage us to formulate the circadian rule as follows: In light-active animals spontaneous frequency, $\alpha:\rho$-ratio, and total activity increase with increasing intensity of continuous illumination. The opposite applies to dark-active animals. The rule makes no statements about the absolute values of the spontaneous frequency. The original concept that in light-active animals the period should be shorter than 24 hours in *LL* and longer in *DD*—as it is in finches and starlings—must not necessarily be generalized. It may be that there are species whose natural period is always longer or shorter than 24 hours (as the hamster seems to indicate). At any rate, theoretically there is no need for crossing the line which represents a free running period of 24 hours. Synchronization and phase-control will also be possible—at least in a model as shown by Wever [31]—if an organism has periods only longer or shorter than 24 hours within the whole range from lowest to highest intensities of illumination. This does not mean that such a possibility must be realized in nature. We rather expect that normally the free running period in *LL* has a trend from less to more than 24 hours with increasing or decreasing light intensity.

There is one other conclusion which may be derived from the results represented in Fig. 8. If level of excitement and spontaneous frequency are in any way related to each other, then one could expect changes of frequency also if the level is changed by any other means than by light. Temperature has a noteworthy effect on the level as measured by activity. Several experiments have been carried out to study the influence of temperature on the spontaneous frequency. The

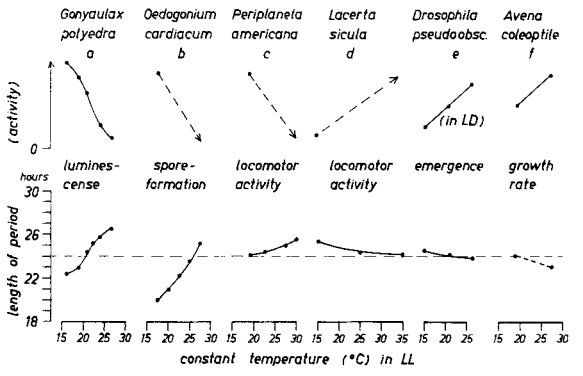

FIGURE 9. Total amount of "activity" and spontaneous period in constant environmental conditions, depending on temperature. *a*) Hastings and Sweeney [31]; *b*) Bühnemann [33]; *c*) Bünning [34]; *d*) Hoffmann [35]; *e*) Pittendrigh [36]; *f*) Ball and Dyke [37].

TABLE 2. PERIOD (τ) AND ACTIVITY-TIME (α = TIME DURING WHICH LUMINESCENCE IS ABOVE A THRESHOLD) IN *Gonyaulax* IN CONSTANT CONDITIONS WITH 5 LEVELS OF CONSTANT TEMPERATURE (T)

T, C.°	τ, hours	α, hours
16.0°	22.8	22.8
19.0°	23.0	16.4
22.0°	25.3	14.7
23.0°	25.7	8.7
26.8°	26.5	6.5

TABLE 3. PERIOD (τ) AND ACTIVITY-TIME (α) IN LIZARDS IN CONSTANT CONDITIONS WITH 3 LEVELS OF CONSTANT TEMPERATURE (T)

T, C.°	τ, hours	α, hours
16°	25.25	5.0
25°	24.34	7.3
35°	24.19	12.7

results seem to corroborate the hypothesis: Organisms which become excited (or more active) by an increasing temperature shorten their period simultaneously; organisms whose activity is depressed by a higher temperature lengthen their period (Fig. 9). The period of 5 organisms mentioned in Fig. 9 has been measured exactly. In the case of *Avena* it was only noted that the period at 27° with respect to 17° "was unaffected, or, if a slight shortening occurred at the higher temperature, it was not more than one hour in 24" [37]. Activity was measured quantitatively only in a few organisms; but there is no doubt in any case about the direction into which the level of excitement (or the amount of activity) is changed with increasing temperature (dotted lines in Fig. 9). It stands to reason that the hypothetical relation as indicated in the diagrams of Fig. 9 needs further examination. That the hypothesis may be right is in addition supported by two of the 6 organisms. In these two cases, the $\alpha:\rho$-ratio varies with increasing temperature toward a direction one would expect from the rule: a) With respect to *Gonyaulax*, this can be computed from data published by Hastings and Sweeney, using their Fig. 1 [32]. If one takes cipher 1 on the ordinate scale as a threshold of luminescence above which all figures are included in the "activity-time," one gets the results shown in Table 2; b) Less doubtful are figures Hoffmann could get in measuring the activity of lizards (Table 3). In accordance with the rule, *Gonyaulax* shortens activity-time and lizards lengthen it with increasing temperature. Clearly, this test is only meaningful in a range of temperature wherein the animals increase their activity with increasing temperature. Only in this range the organism may be called "warm-active" as it is "light-

active" in the sense of increasing activity with increasing intensity of illumination. In both cases there is an "optimum" above which activity decreases; then the organism becomes dark-active and cool-active respectively. Light-active animals will mostly show "warm-activity" and dark-active animals "cool-activity" in testing the animals within the range of intensities in which they normally live. *Gonyaulax* as an exception seems to be light-active and cool-active together. The same may apply to *Saintpaulia ionontha*, as shown by Went, because in a cyclic environment *Saintpaulia* prefer the cool fraction of the temperature cycle coinciding with the light fraction of the light-dark-cycle. Therefore one should expect that *Saintpaulia* would change its spontaneous frequency (if there is one) in opposite directions by increasing light-intensity and by increasing temperature.

I.6. Accuracy of the Clock

If in constant conditions the clock does not run with a period of exactly 24 hours, this may not be "a measurement of inaccuracy of the clock" [38]. Accuracy is measured by how precisely the clock keeps a certain frequency. For this purpose one has to measure how much each single period deviates from the average of all measured periods. Usually this measurement is given as standard deviation. To compare the accuracies of several frequencies, it is preferable to express the standard deviation in per cent of the mean value. This has been done in Fig. 10 with period, activity-time, and rest-time of 4 chaffinches. The activity of the birds was measured in constant illumination with 4 different intensities. Each point of the diagram represents the standard deviation in one frequency of one bird during at least 10 days. In some cases, if the spontaneous frequency has changed from one to another value during the same illumination (compare Fig. 3), the standard deviation has been computed for both frequencies. Each point in the upper-most diagram corresponds with a point in the middle (activity-time) and in the lower-most (rest-time).

At first sight it is evident from Fig. 10 that the accuracy of the clock varies with the frequency and that there is a difference of accuracy between activity-time and rest-time. Judging the diagram concerning period, one can say that the clock runs the less accurately the shorter the period; most precise is the clock at a spontaneous period which corresponds to that of earth-rotation. Whether it becomes again less accurate toward longer periods (that means: at a low frequency in very dim light) is still not cleared up, but indicated by few points on the right end of the diagram. Activity-time (diagram in the middle of Fig. 10) remains nearly equally precise within a large range of frequencies; however, accuracy here becomes also worse with increasing length of activity-time (which means increasing frequency). Thirdly, the rest-time

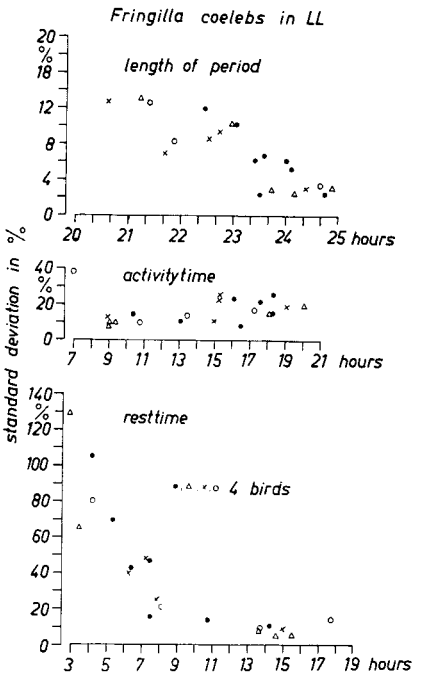

Figure 10. Standard deviation from mean value of period, activity-time and rest-time, depending on spontaneous frequency in *LL* with varying intensity of illumination. Each point represents the average of several periods in one bird.

becomes quickly less and less precise the more rest-time shortens (with other words: the faster the clock runs). Above all, in the average of all 4 birds and all frequencies, activity-time has a smaller standard deviation than rest-time. Regarding this, one other observation may be important. Analyzing each single period with respect to activity-time and rest-time, one finds a strong negative correlation between these two components. Whenever activity-time is longer than in the average, the following rest-time is shorter and vice versa. From these two facts one could conclude that the clock regulates its period especially by keeping constant activity-time and by correcting errors via rest-time. But this explanation, although thinkable, is only one of several possibilities. If only the onset of activity is more precisely fixed than the end (as for instance the firing potential as compared with the cutoff potential of a brush discharge in an electronic circuit), the same negative correlation between activity- and rest-time would happen. In this case, the clock will keep its accuracy by correcting period instead of activity-time, which seems to be more likely.

II. EXOGENOUS COMPONENTS

II.1. General Remarks

The periodical factors of the environment, which we call Zeitgeber, have several effects:

a) Zeitgeber synchronize a circadian periodicity

with the environment;

b) Zeitgeber synchronize several individuals of one species and keep them in phase;

c) Zeitgeber synchronize several circadian clocks in an organism, if it has more than one;

d) Zeitgeber synchronize by determining phase;

e) Zeitgeber influence the circadian pattern.

Statement a) is right by definition. Statement b) implies that all individuals have the same sensitivity to the Zeitgeber; if this were not so, the individuals would not be in phase (compare chapter II,4), and one would not find what one calls a species-pattern (compare chapter II.6). Statement c) tackles the question of endodiurnal organization as Pittendrigh calls it. Most important is statement d) as a consequence of statement a); it is related to, but not identical with, the last statement e) (compare again II.4 and II.6).

With respect to rhythms, synchronization means that at least two rhythms are in synchrony with each other. Therefore, only such factors of the environment which pass off periodically can operate as a Zeitgeber. One single event can influence the phase of a rhythm; therefore, a group of rhythms running out of phase with each other can be pushed into phase for a short time by one single event. But a single event can never synchronize continuously. Therefore, we may not agree with such a statement as: single isolated events are "an important class of time-givers" [39]. Only repeated single events can operate as a Zeitgeber. The first more detailed definition of Zeitgeber says: "Es kann sich ebenso gut um diskontinuierliche (z. B. ein Tonsignal alle 24 Stunden) wie um kontinuierliche periodische Vorgaenge handeln (z. B. taeglicher Temperaturgang) bzw. um den regelmaessig wiederkehrenden Uebergang eines Zustandes in einen anderen (Hell-Dunkel-Wechsel)" [4]. That means: A Zeitgeber can be represented by a) one short signal each 24 hours, b) a continuously changing factor such as the daily course of temperature, or c) alternating steady state conditions, e.g., alternating light and darkness. These are three thinkable types of Zeitgeber. Whether they all are effective in practice has to be tested.

The question, which parameter of a Zeitgeber acts as the really efficient one, is more difficult to answer. Two possibilities which have to be considered have been discussed together with the definition of Zeitgeber cited above. The difference between both of them is easy to explain by using alternating light and darkness as a Zeitgeber. Case one: The steady state itself is effective, that means: the whole light-time and/or the whole dark-time. Case two: Only the transitions from one to the other state are effective, that means: under natural conditions dawn and dusk. Combinations of both cases may also be possible. Following a suggestion made by Wever [31], the first type may be called proportional effect and the second type differential effect (Fig. 11). Proportional effect means: The effect

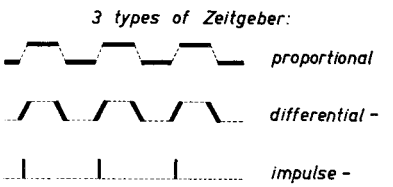

FIGURE 11. Simplified scheme of 3 possible types of effective Zeitgeber components.

is proportional to the reaction which the stimulus of the Zeitgeber in its steady state causes in the organism; the effect continues steadily as long as the intensity of the stimulus is unchanged. Differential effect means: the effect depends only on changing factors of the environment; their steady state has no effect. A special type of Zeitgeber not realized in nature is that of repeated single events. They may be called impulse Zeitgeber. Impulse Zeitgeber are a borderline case of Zeitgeber with two differential effects, so far as the change from one steady state to another one is followed by the reverse change to the original state within an extremely short time.

To become effective, a Zeitgeber must influence the phase of the organism. There are two possibilities to shift the phase of an oscillation. a) Each sudden downward or upward step of the level results in a phase-shift. The amount of the shift depends on the phase at which the level has been changed and on the difference between the two levels. In at least two phases there will be no phase-shift from changing the level. b) Each change of speed for a given fraction of period causes a phase-shift; the amount of the shift depends on the rate of changed speed and how long it remains changed. The question now arises by what means the proportional and differential stimuli of a *LD*-Zeitgeber shift the phase of an organism. As mentioned above, the proportional effect may be described as the influence on speed. The longer the system runs faster or slower—i.e., the longer light-time or dark-time—the greater the advance or the delay respectively. On the other hand, the differential Zeitgeber is effective during a nearly immeasurable short time in an artificial lighting regimen. It would be meaningless to speak of an altered angular velocity during this very short time. The effect rather immediately is a phase shift. The amount of the shift depends on the value of the differential coefficient. Whether only one or both of these types of Zeitgeber also influence the level of excitement can so far not be derived from our knowledge. How important this question may be with respect to all problems of phase-control will be discussed by Wever [31].

Until now there is no decision possible whether the natural Zeitgeber operate proportionally or differentially. Probability seems to indicate that in most of all organisms both types of Zeitgeber are cooperating. Nevertheless, it is quite possible that one species

follows mainly the differential principle while another one (e.g., mice?) is synchronized more proportionally (compare II.4). Theoretically, an impulse Zeitgeber should also be possible. Curiously enough, this case never has been tested experimentally. A few observations seem to contradict it. If light-time or dark-time of an artificial *LD*-regimen with a total period of 24 hours is shortened extremely—that means to less than one hour—some organisms lose synchronization and run with their own frequency. These results may not necessarily prove that an impulse Zeitgeber is ineffective on principle. One has rather to test whether in these cases the differential impulse was not only too weak and thus unable to counteract the strong influence on speed of the extremely long light-time (or dark-time). Obviously, an impulse Zeitgeber with a 24-hour period must be made the stronger the more the other conditions (e.g., intensity of light during *L* and length of *L*) push off the free running period of the organism from 24 hours (compare II.2).

To become effective, a Zeitgeber must send out stimuli, and to become synchronized, the organism must be sensitive to these stimuli. A periodically varying sensitivity or responsiveness is one of the inevitable characteristics of each self-sustained oscillation. An excised frog heart is often used to demonstrate the rhythmically varying threshold to electric stimulation. Also, diurnal changes of responsiveness have been shown, mostly by using photoperiodic effects as an indicator [39a], but also true sensitivity-curves by measuring thresholds in men [40]. How important, however, such a rhythm may be with regard to phase-control became only evident in the excellent experiments wherein phase-shifts resulting from single light-signals have been measured [14, 17, 39, 41, 42]. The results demonstrate directly the circadian periodicity of responsiveness. Similar facts have been found out by using temperature shocks [44, 45].

The circadian response-curves, when measured by using extremely short flashes of light as perturbations, are the most clear expression of the differential effect of a Zeitgeber. Clearly enough, proportional effects of a Zeitgeber can not be derived from such a curve. If, on the other hand, light-signals have been used with a measurable duration of time, the resulting phase-shift is the combination of two differential and one proportional stimuli. It is very difficult to decide whether the phase-shift was caused by one or both of the differential stimuli, by the proportional stimulus, or by all three together. In increasing the duration of the light-signal the proportional effect must become greater with respect to that of the two differential stimuli. That means: The greater the distance between light-on and light-off the clearer the proportional effect if there is one. Therefore *LL* shows—as mentioned above—the proportional effect of *L* without any additional differential stimuli (Fig. 4). Considering once more Fig. 9, one can guess that temperature only in *Gonyaulax* has a remarkable proportional effect but not in the five other organisms. A few difficulties may be briefly mentioned. If proportional effects have been proven by measuring τ in different steady state intensities of *LL*, this does not necessarily mean that also in *LD* the proportional stimulus becomes effective; there may be something like a "blocking" effect from the preceding differential stimulus. If, on the other hand, τ does not change with increasing intensities of illumination in *LL*, this does not necessarily mean that there is no Zeitgeber-effect; if different light-intensities are combined with different levels, alternating changes between two intensities are differentially effective.

II.2. Testing a Zeitgeber

An environmental periodicity may be called a Zeitgeber only if the organism synchronized with the environment possesses a circadian periodicity. Studying the mechanism of Zeitgeber therefore presumes organisms whose spontaneous frequency has been demonstrated. In testing whether an environmental periodicity operates as a Zeitgeber on such an organism or not, three types of experiments may be considered:

a) Catching the free running clock. In constant conditions the spontaneous frequency of an organism is measured; afterward the Zeitgeber to be tested is added for several periods and then again the conditions are kept constant. An example of such an experiment is given in Fig. 2.

b) Phase-shift. The periodical factor of the environment the organism already is synchronized with has to be shifted suddenly by a certain phase-angle. If the Zeitgeber is fully effective, the organism should follow the shift within a few periods and should regain the original phase-relation to the Zeitgeber. An experiment in this line carried out with finches in *LD* (12:12, 400 lux: 0.4 lux) is shown in Figs. 12 and 13. The 12-hour shift is done once by doubling light-time and once by doubling dark-time. Within about 4 periods, the birds are always resynchronized. During synchronizing the amplitude is damped (Fig. 12). The phase-angle-difference between organism and Zeitgeber becomes evident in Fig. 13. In this diagram again the sequence of days (as the independent variable) is represented by the abscissa; onsets and ends of activity are given on the ordinate in Central European time. Better than with words, the picture explains the mechanism of shifting phase: By doubling light-time, the period of the bird is shortened, by doubling dark-time, it is lengthened. The opposite to this one would expect in dark-active animals.

c) Varying frequency. By use of an effective Zeitge-

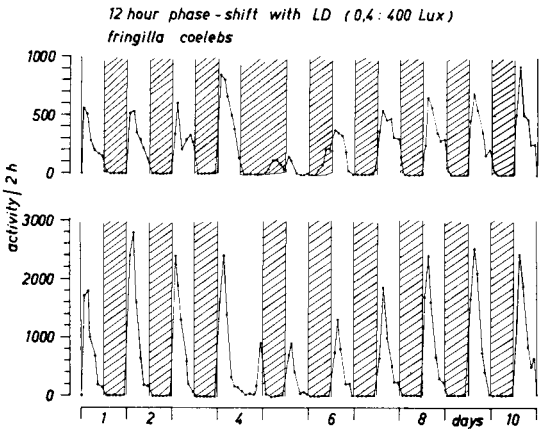

FIGURE 12. Activity of chaffinch in *LD* (12:12). Phase-shift of the Zeitgeber for 12 hours by doubling light-time (below) or dark-time (above).

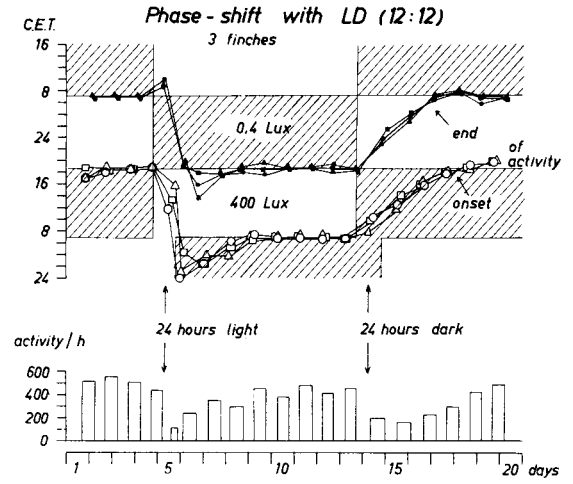

FIGURE 13. Phase-shift experiment with *LD* (12:12, 400 lux: 0.4 lux). Onset and end of activity of three chaffinches, given for each consecutive day (abscissa) on the ordinate-scale in Central European time.

ber it must be possible to entrain an organism to different frequencies within certain limits. Examples of such experiments are presented in Figs. 15 and 17.

The original definition of Zeitgeber says that all environmental factors may be considered "to whose stimuli the organism is sensitive" [4]. Recently some authors incline more toward the opinion that Zeitgeber are more specific. Periodicities of temperature and of illumination are generally accepted as Zeitgeber. On the other hand, few experiments have been published indicating that a periodic feeding or irritation by noise may be no Zeitgeber [14, 39]. But there are doubts whether these results are fully convincing. From experiments just carried out at Heidelberg we must conclude that birds running with their spontaneous frequency in *LL* (0.4 lux) can be synchronized with 24 hours if they are fed for 12 hours and kept without food for the following 12 hours. The same seems to be true if an unspecific noise for 12 hours alternates with 12 hours of silence. The experiments are still in progress; therefore, a final answer is impossible. At all events, these unspecific Zeitgeber if ever effective become ineffective if the continuous illumination is increased to higher intensities. As mentioned above, a relatively weak 24-hour Zeitgeber can not catch a free running period deviating too far from 24 hours.

Obviously, not all possible Zeitgeber are equally effective. Light and temperature are the main parameters of the environment to which the organisms have been adapted. The "strength" of a Zeitgeber depends on several elements, some of which may be mentioned here: sensitivity of the organism to the stimulus represented by the Zeitgeber; time-ratio and intensity-ratio of the two fractions of the Zeitgeber; absolute value of stimulus-intensity during the whole period of the Zeitgeber (e.g., intensity of illumination as well during light-time as during dark-time); general circumstances (e.g., degree of constant temperature in *LD*, functional state of the organism, and so on).

II.3. Masking Factors and Zeitgeber

Proportional and differential Zeitgeber are only effective if the respective amplitude is big enough. In *LD* there must be a certain difference in intensity of illumination between *L* and *D*. Moreover, not only the difference, but also the absolute values, of intensity are important. An amplitude of 200 lux may represent an excellent Zeitgeber, if *D* means "total darkness." The same amplitude operating between 4 lux and 204 lux may be on the borderline to become ineffective (compare Fig. 16). With respect to this, it may be right to carry out experiments in *LD* with a *D* of "total darkness." But in studying some special qualities of the Zeitgeber-mechanism, e.g., phase-control, this may be wrong. In nature, night never means a darkness as we can perform it in the laboratory. Experiments with an "absolute" dark *D* can therefore be misleading, especially if optical orientated animals are used. An example is presented in Fig. 14, published in an earlier paper of our own [46]. A greenfinch, kept in an artificial 24-hour day with 10, 8, 6, or 4 hours of light, starts and finishes its activity exactly with "light-on" and "light-off." But if there had been an illumination with an intensity of only 0.1 lux during dark-time, quite another result may have happened, (compare Figs. 16 and 19). Figure 14 is shown here to stress that certain (sometimes overlooked) experimental conditions can obscure the real Zeitgeber-mechanism. We may call them masking conditions [47]. Each experiment stays as a question, and the organism should be free to answer as it inclines; this was not the case in the experiment drawn in Fig. 14. To use other words: Especially in experiments with Zeitgeber, the conditions should

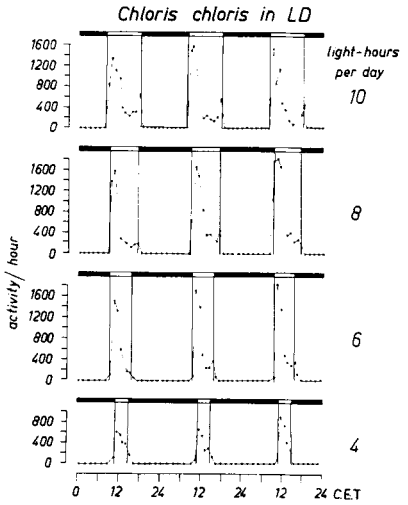

FIGURE 14. Activity of a greenfinch in *LD* (24-hour period) with 10, 8, 6 and 4 hours of light alternating with total darkness. Misleading experiment (compare text). (From Z. Tierpsychol. *12* (1955) Parey-Verlag. Berlin.)

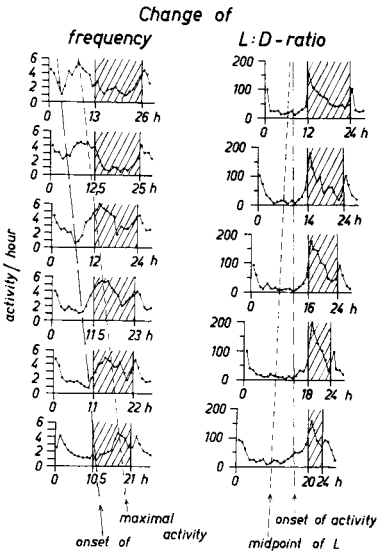

FIGURE 15. Pattern of activity of mice in *LD*, averaged on 3 individuals and at least 6 periods in each diagram. Left column: Period of the Zeitgeber varying from 26 to 21 hours; *L:D*-ratio constant (50% of period each). Right column: Varying *L:D*-ratio of the Zeitgeber, period remaining constant (24 hours).

not be masking (inhibitive) but as "permissive" as possible.

II.4. The Phase-Angle

Synchronization takes place by determining the phase. That means: An organism synchronized with a Zeitgeber keeps a definite phase-angle-difference to the Zeitgeber. In order to measure the phase-angle-difference, one must select reference points as well in the period of the Zeitgeber as in that of the organism. For this purpose several possibilities are on hand which may be discussed in the light of Fig. 15. The diagrams of the two columns show the activity of mice in *LD*, each curve averaged on three individuals and several periods. The right side diagrams are the results of experiments [48] in which the *LD*-period was always 24 hours; the *L:D*-ratio has been varied within the limits 12:12 and 20:4. The left column shows results of experiments in *LD* with a period varying from 26 to 21 hours; *L* and *D* have been kept on equal lengths.

The right column shall be discussed first. Two main reference points of the Zeitgeber are "dawn" and "dusk." The midpoints, however, of light-time or dark-time as representatives of both time spans could also be used. The biological period offers the peaks of activity or the minima (= onsets) for reference. In both cases it is not clear a priori which reference points are more meaningful or more important for phase-control. In examining the diagrams, it is evident that the peaks keep the same phase-angle-difference with respect to dawn and dusk in all conditions, but a changing one with respect to the midpoints. The onsets are not as clear. But also here, the dashed line running parallel to dawn, seems to fit the onsets with the exception of only the lower-most diagram. Is there any possible conclusion from these facts? If dawn and dusk both had a differential effect on the organism, one would expect that the phase should be bound to a point between them. The result would be that onsets as well as peaks of activity in the right column of Fig. 15 would be kept parallel to the midpoints. If, on the other hand, in this case the Zeitgeber would be a proportional one, the increasing influence on angular velocity of the increasing light-time should be compensated by a phase-shift of the organism with respect to light- and dark-time. Therefore, in the case of a proportionally operating Zeitgeber, the organism would change its phase-angle-difference to the midpoints. As the mice do this, whether one watches peaks or onsets, we may predict that *LD* in mice operates more as a proportional than as a differential Zeitgeber. On the other hand, it is evident that both dawn and dusk are followed by an increasing activity; that may be rather a differential effect of the Zeitgeber on pattern.

In varying the frequency of the Zeitgeber (left column of Fig. 15), there is a clear and big change in the phase-angle-difference of the organism whatever may be used for reference. If the period of the Zeitgeber is longer than 24 hours, the phase of the organism is advanced; if the period is shorter, the phase is delayed. In an artificial 26-hour day, activity starts at the very beginning of light-time; the mice are completely light-active. In the 21-hour day, the onsets have been shifted toward the beginning of dark-time; the mice are dark-active. There is one more interesting fact. In the 26-hour day as well as in the 21-hour day, the mice perform nearly the same amount of total

activity. That means: in mice, total activity is nearly independent of whether the mice are active in light or in darkness. This fact, corresponding with the results of experiments in *LL* of different light-intensities (Fig. 8), indicates again that phase-control in mice is done mostly by influencing angular velocity instead of level of excitement [31.] This may be taken as one more evidence for *LD* operating mainly as a proportional Zeitgeber on mice.

Phase does not depend only on the *L*:*D*-ratio or on *LD*-frequency. Intensity of illumination in *L* and *D* is also important. As shown in Fig. 13, activity-time of chaffinches in *LD* (12:12) may be identical with the light-time, even if *D* does not mean "total darkness" but dim illumination with an intensity of 0.4 lux. One gets quite another picture if the intensity of illumination during *D* is increased to 4 lux (Fig. 16, upper diagram). Activity now starts in the middle of dark-time; it ends as in the experiment shown in Fig. 13 together with light-off. Under the influence of 4 lux during *D* instead of 0.4 lux, the chaffinch keeps quite another phase-angle-difference to the Zeitgeber using onset of activity for reference; activity-time is now longer by 50 per cent. That an alternating *LD* with 200 lux during *L* and 4 lux during *D* is efficient as a Zeitgeber is shown by the phase-shift executed with the Zeitgeber. After doubling light-time, two chaffinches are resynchronized within few periods and keep then the same phase-angle to the Zeitgeber as before. A third bird behaves in a different way (Fig. 16, lower diagram). This bird is remarkable from the beginning, because instead of being synchronized, it runs during the first 4 days of the experiment with a spontaneous period of about 16 hours through the Zeitgeber and is caught not earlier than on the fifth day. But even then, its

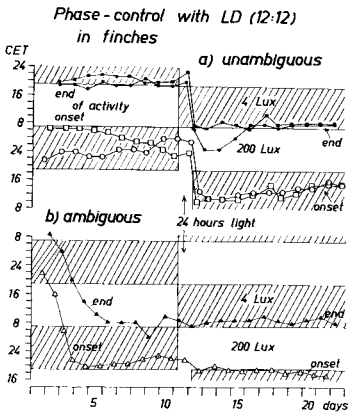

FIGURE 16. Chaffinches in *LD* (12:12) with 200 lux in *L* and 4 lux in *D*. Onset and end of activity in Central European time on the ordinate. Phase-shift of the Zeitgeber on the 11th day by doubling light-time. Upper diagram: Effective Zeitgeber in two finches, unambiguous phase-control. Lower diagram: Catching the spontaneous frequency of one bird by a weak Zeitgeber with ambiguous phase-control.

phase is quite abnormal: activity starts when the illumination changes from 200 lux to 4 lux, and activity ends with the beginning of bright light. After the Zeitgeber has been shifted, the bird is still synchronized; but now, the phase-angle-difference between bird and Zeitgeber is reversed by 180°. The diagrams of Fig. 16 are an example of the "strength" of a Zeitgeber depending on the absolute value of stimulus-intensity in both of its time-fractions. Using a Zeitgeber on the borderline between being effective or ineffective, synchronization may still be possible but phase-control becomes ambiguous.

One more conclusion seems possible by observing the diagrams in Fig. 16. As other experiments have shown, the free running period of a chaffinch is shorter than 24 hours in *LL* with an intensity of 200 lux as well as with an intensity of 4 lux. If the *LD*-Zeitgeber would operate on birds only as a proportional one—that means: only by influencing angular velocity—one could not expect synchronization with 24 hours by an alternating illumination with intensities of 200 lux and 4 lux. Therefore, *LD* can not operate as a pure proportional Zeitgeber on birds; there must be a more or less strong differential effect.

II.5. Comparison of Phases in Different Organisms

From the experiments described in the foregoing chapter one concludes that phase is a function of at least three parameters of a Zeitgeber: *L*:*D*-ratio, *LD*-frequency, and intensity of illumination in *L* and in *D*. (This applies also to other Zeitgeber respectively, but only the relationships in *LD* are clarified). It may be interesting enough to survey phase-relation of different organisms in different experimental conditions. Evidently it could be that each species reacts differently if one changes one or more of the three parameters of a Zeitgeber mentioned above. Surprisingly enough this is not the case as a survey on literature shows. All organisms so far tested behave the same in experiments with varying *LD*-ratio or *LD*-frequency. Figures 17 and 18 contain 10 examples each, representing phase-control in both types of experiments. In varying frequency (Fig. 17) all organisms shift their phases toward the same direction: with high frequencies the phases are delayed; with low frequencies they are advanced with respect to the phase-relation at a 24-hour period. If frequency remains unaltered and only the *L*:*D*-ratio is varied (Fig. 18), the resulting phase-shifts remain far less. In some organisms phase tends to stay more closely to the midpoints, in others more to dawn or dusk. In general, in light-active animals phase stays more or less parallel to "light-on," in dark-active animals parallel to "dark-on." As mentioned above, this is no strong indication of a differential effect, but rather more of a proportional one. (The figures for

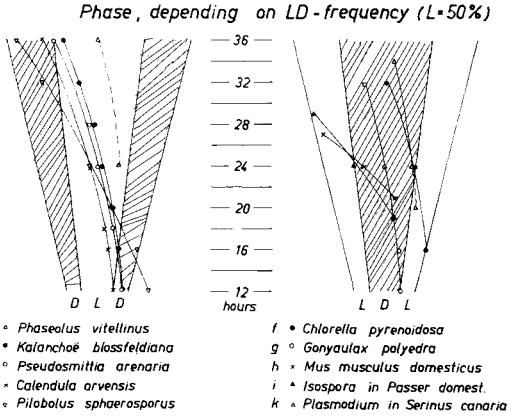

FIGURE 17. Phase in *LD*, depending on *LD*-frequency (*L* and *D* 50% of period each). *a*) Leaf movements, Pfeffer [19]; *b*) Opening of flowers, Bünsow [43]; *c*) Emergence, Remmert [50]; *d*) Flowers, Stoppel [51]; *e*) Spore formation, Uebelmesser [52]; *f*) Cell division, Lorenzen [53]; *g*) Luminescence, Hastings and Sweeney [54]; *h*) Maximal activity, Tribukait [49]; *i*) Isospora reproduction, Boughton [55]; *k*) Plasmodium reproduction, Boyd [56].

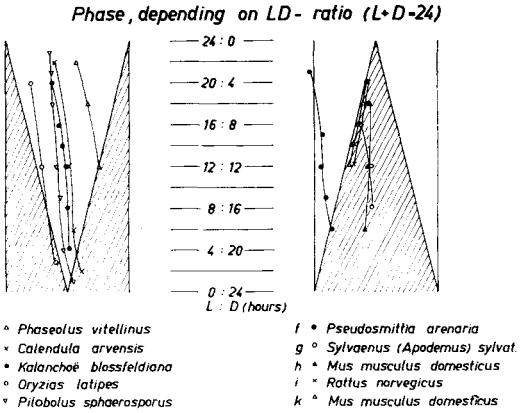

FIGURE 18. Phase in *LD*, depending on *L*:*D*-ratio (*L* + *D* = 24 hours). *a*) Leaf movements, Pfeffer [19]; *b*) Opening of flowers, Stoppel [51]; *c*) Flowers, Bünsow [43]; *d*) Oviposition, Egami [57]; *e*) Spore formation, Uebelmesser [52]; *f*) Emergence, Remmert [50]; *g*) Peak of activity, Miller [58]; *h*), *i*) and *k*) Peak of activity.

Sylvaenus are derived from very sketchy data and therefore not conclusive.)

The differences in the amount of phase-shift in the two experiments as shown in Figs. 17 and 18 may be partly the result of one clear fact: In varying the frequency of the Zeitgeber, the two effective parameters of the Zeitgeber are moved in the same direction. If frequency remains constant and *L*:*D*-ratio varies, the differential and proportional parameters of the Zeitgeber are moved in opposite directions. Therefore, the Zeitgeber effects are summated in the first case, but are conflicting in the other one. The possible consequence of the second fact on some problems of

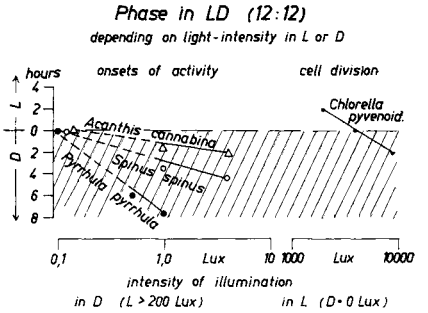

FIGURE 19. Phase in *LD*, depending on intensity of illumination in *L* or *D*. Ordinate: Hours before or after "light-on." Figures for *Chlorella* from Pirson and Lorenzen [59].

seasonal photoperiodism will be discussed elsewhere [31].

The influence of intensity of illumination on phase has not been studied in detail. A few results seem to indicate that also here divers organisms behave similarly. In the left diagram of Fig. 19, the onsets of activity of three different birds are marked on the ordinate; hour '*O*' corresponds with "light-on." In all experiments, the intensity of illumination during *L* was the same (200 lux); the very low intensity during *D* was varied. In all three species, activity starts the earlier, the brighter the light in *D*. The results may be compared with experiments carried out on *Chlorella* [59]. In this case, dark-time was kept absolutely dark while the intensity of illumination during light-time was varied. In these experiments, the phase of the organism was also advanced with an increasing intensity of illumination.

II.6. Coaction of Endogenous and Exogenous Components

As mentioned in chapter II.1, and in relation to Fig. 15, Zeitgeber do not only synchronize but can also influence the pattern of circadian periodicity. More important than these external influences are the endogenous ones. The special structure of an organism determines whether it becomes in *LD* light- or dark-active, and causes, moreover, some single events during the total period. Two examples may illustrate such species and individual patterns [60]. Differences in circadian pattern have been known for a long time. In Fig. 20 the patterns of three mainly dark-active species are drawn. In these diagrams, variations in the total amount of activity have been excluded by computing all figures as per cent of the daily mean. Each curve represents the average of one individual on several days. The three curves of the three individuals in one diagram are close to each other in their respective shapes, thus providing a clear species pattern. The pattern of one species is clearly separated from those of the two others.

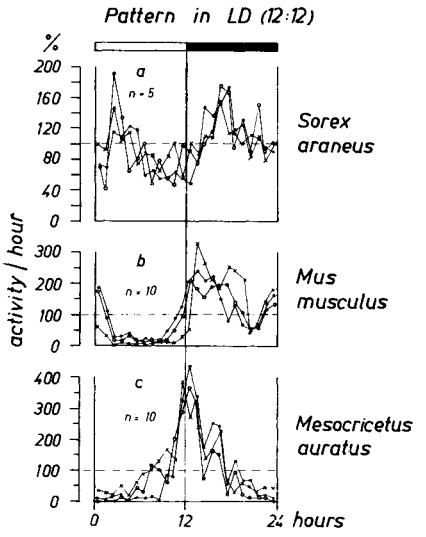

FIGURE 20. Species-pattern of activity in *LD* (12:12). Three individuals in each species. Each pattern-diagram represents the average for 5 days (*Sorex*) or 10 days (*Mus* and *Mesocricetus*) of one individual. Figures for *Sorex* from Crowcroft [61].

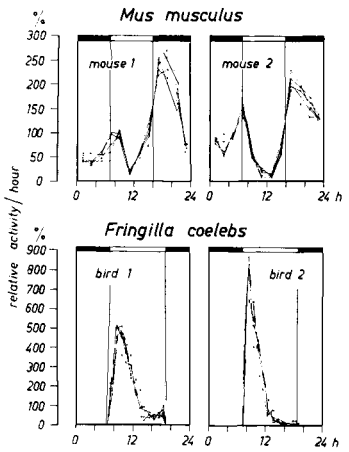

FIGURE 21. Individual pattern of activity in two mice (*LD* 9:15) and two greenfinches (*LD* 12:12). Five consecutive days superimposed in each diagram. (100% of ordinate = average activity per 24 hours.)

Although generally several individuals in one species have similar patterns, it is nevertheless possible to work out individual differences. In Fig. 21 the individual patterns of two mice and two birds are shown, all measured in artificial *LD*. The pattern of one individual remains nearly unaltered during 5 consecutive days whose activity-figures are superimposed in one diagram. The differences between the patterns of two individuals of one species are statistically significant [60]. Inter-individual differences in sensitivity to light may be one of the elements responsible for the special individual pattern; they also cause the individually different time-lags between dawn or dusk, and onset of activity (individual phase-angle-differences) [39, 62, 63]. Obviously, the individual as well as the species pattern will not remain constant for too long a time. It is known that the pattern varies as the organism grows up, and also in the adult we may expect variations of the pattern at least from season to season [23]. The migrating birds offer a good example in alternating between light- and dark-activity. Nevertheless, we can speak of a species-pattern as well as of an individual pattern so long as the conditions (inside and outside the organism) remain comparable.

REFERENCES

1. ASCHOFF, J. 1959. Zeitliche Strukturen biologischer Vorgaenge. Nova acta Leopoldina, *21:* 147–177.
2. HALBERG, F. 1959. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine. Pp. 803–878. *Photoperiodism and Related Phenomena in Plants and Animals*, ed. Withrow. Washington: A.A.A.S.
3. ASCHOFF, J. 1951. Die 24-Stunden-Periodik der Maus unter konstanten Umgebungsbedingungen. Naturwiss., *38:* 506–507.
4. ——. 1954. Zeitgeber der tierischen Tagesperiodik. Naturwiss., *41:* 49–56.
5. HALBERG, F., M. B. VISSCHER, and J. J. BITTNER. 1954. Relation of visual factors to eosinophil rhythm in mice. Am. J. Physiol., *179:* 229–235.
6. BRUCE, V. G., and C. S. PITTENDRIGH. 1957. Endogenous rhythms in insects and microorganisms. Am. Natural., *91:* 179–195.
7. PITTENDRIGH, C. S. 1958. Perspectives in the study of biological clocks. Pp. 239–268. *Symposium on Perspectives in Marine Biology*. Berkeley: Univ. of California Press.
8. PFEFFER, W. 1909. Untersuchungen ueber die Entstehung der Schlafbewegungen der Blattorgane. Abhandl. saechs. Akad. Wiss., math.-physik. Kl., *30:* 257–272.
9. BROWN, F. A., JR. 1957. Biological chronometry. Am. Natural., *91:* 129–133.
10. ——. 1957. The rhythmic nature of life. Pp. 287–304. *Advances in Invertebrate Physiology*. Univ. Oregon Publ.
11. ——. 1959. Living clocks. Science, *130:* 1535–1544.
12. WEBB, H. M., and F. A. BROWN, JR. 1959. Timing long-cycle physiological rhythms. Physiol. Rev., *39:* 127–158.
13. ASCHOFF, J., and J. MEYER-LOHMANN. 1954. Die Schubfolge der lokomotorischen Aktivitaet bei Nagern. Pflügers Arch., *260:* 81–86.
14. RAWSON, K. 1956. Homing behavior and endogenous rhythms. Ph.D. Thesis, Harvard Univ.
15. ASCHOFF, J., and J. MEYER-LOHMANN. 1954. Die 24-Stunden-Periodik von Nagern im natuerlichen und kuenstlichen Belichtungswechsel. Z. Tierpsychol., *11:* 476–484.
16. JOHNSON, M. 1939. Effect of continuous light on periodic spontaneous activity of white-footed mice (*Peromyscus*). J. Exper. Zool., *82:* 315–328.
17. HASTINGS, J. W., and B. M. SWEENEY. 1958. A persistent diurnal rhythm of luminescence in *Gonyaulax polyedra*. Biol. Bull., *115:* 440–458.
18. HOFFMANN, K. 1960. Versuche zur Analyse der Tagesperiodik I. Der Einfluss der Lichtintensitaet. Z. vergl. Physiol. (in press)

19. PFEFFER, W. 1915. Beitraege zur Kenntnis der Entstehung der Schlafbewegungen. Abhandl. saechs. Akad. Wiss., math.-physik. Kl., *34:* 1–154.
20. ASCHOFF, J. 1952. Frequenzaenderungen der Aktivitaetsperiodik bei Maeusen im Dauerlicht und Dauerdunkel. Pflügers Arch., *255:* 197–203.
21. ——. 1958. Tierische Periodik unter dem Einfluss von Zeitgebern. Z. Tierpsychol., *15:* 1–30.
22. ——. 1959. Periodik licht- und dunkelaktiver Tiere unter konstanten Umgebungsbedingungen. Pflügers Arch., *270:* 9.
23. ——. 1957. Aktivitaetsmuster der Tagesperiodik. Naturwiss., *44:* 361–367.
24. PITTENDRIGH, C. S., and V. G. BRUCE. 1957. An oscillator model for biological clocks. Pp. 75–109. *Rhythmic and Synthetic Processes in Growth.* Princeton Univ. Press.
25. SCHWASSMANN, H., and W. BRAEMER. 1960. Personal communication.
26. ASCHOFF, J., and J. MEYER-LOHMANN. 1954. Angeborene 24-Stunden-Periodik beim Kuecken. Pflügers Arch., *260:* 170–176.
27. HELLBRÜGGE, TH. 1960. The development of circadian rhythms in infants. Cold Spring Harbor Symp. on Quant. Biol. Vol. 25.
28. HOFFMANN, K. 1957. Angeborene Tagesperiodik bei Eidechsen. Naturwiss., *44:* 359–360.
29. ——. 1959. Die Aktivitaetsperiodik von im 18- und 36-Stunden-Tag erbrueteten Eidechsen. Z. vergl. Physiol., *42:* 422–432.
30. ASCHOFF, J. 1955. Tagesperiodik von Maeusestaemmen unter konstanten Umgebungsbedingungen. Pflügers Arch., *262:* 51–59.
31. WEVER, R. 1960. Possibilities of phase-control, demonstrated by an electronic model. Cold Spring Harbor Symp. on Quant. Biol. Vol. 25.
32. HASTINGS, J. W., and B. M. SWEENEY. 1957. On the mechanism of temperature independence in a biological clock. Proc. Nat. Acad. Sci., *43:* 804–811.
33. BÜHNEMANN, F. 1955. Das endodiurnale System der Oedogonium-zelle III. Ueber den Temperatureinfluss. Z. Naturforschg., *10b:* 305–310.
34. BÜNNING, E. 1958. Ueber den Temperatureinfluss auf die endogene Tagesrhythmuk, besonders bei *Periplaneta americana.* Biol. Zbl., *77:* 141–152.
35. HOFFMANN, K. 1957. Ueber den Einfluss der Temperatur auf die Tagesperiodik bei einem Poikilothermen. Naturwiss., *44:* 358–359.
36. PITTENDRIGH, C. S. 1954. On temperature independence in the clock system controlling emergence time in *Drosophila.* Proc. Nat. Acad. Sci., *40:* 1018–1029.
37. BALL, N. G., and I. J. DYKE. 1954. An endogenous 24-hour rhythm in the growth rate of the *Avena* coleoptile. J. Exper. Bot., *5:* 421–433.
38. HASTINGS, J. W. 1959. Unicellular clocks. Ann. Rev. Microbiol., *13:* 297–312.
39. DECOURSEY, P. J. 1959. Daily activity rhythms in the flying squirrel, *Glaucomys volans.* Ph. D. Thesis, Univ. of Wisconsin.
39a. ASCHOFF, J. 1955. Jahresperiodik der Fortpflanzung bei Warmbluetern. Studium generale *8:* 742–776
40. GEHRKE, K. 1956. Tageszeitliche Unterschiede der Gehoersempfindlichkeit. Dr. Dissertation, Univ. Goettingen.
41. PITTENDRIGH, C. S., V. G. BRUCE, and P. KAUS. 1958. On the significance of transients in daily rhythms. Proc. Nat. Acad. Sci., *44:* 965–973.
42. BRUCE, V. G., and C. S. PITTENDRIGH. 1958. Resetting the *Euglena* clock with a single light stimulus. Am. Natural., *92:* 295–306.
43. BÜNSOW, R. 1953. Endogene Tagesrhythmik und Photoperiodismus bei *Kalanchoë blossfeldiana.* Planta, *42:* 220–252.
44. STEPHENS, G. C. 1957. Influence of temperature fluctuations on the diurnal melanophore rhythm of the fiddler crab *Uca.* Physiol. Zool., *30:* 55–69.
45. BÜNNING, E. 1959. Zur Analyse des Zeitsinnes bei *Periplaneta americana.* Z. Naturforschg., *14b:* 1–4
46. ASCHOFF, J., and J. MEYER-LOHMANN. 1955. Die Aktivitaet gekaefigter Gruenfinken im 24-Stunden-Tag bei unterschiedlich langer Lichtzeit mit und ohne Daemmerung. Z. Tierpsychol., *12:* 254–265.
47. FRY, F. E. J. 1947. Effects of the environment on animal activity. Univ. Toronto Stud. Biol. Ser., *55:* 1–62.
48. ASCHOFF, J., and J. MEYER-LOHMANN. 1955. Die Aktivitaetsperiodik von Nagern im kuenstlichen 24-Stunden-Tag mit 6 bis 20 Stunden Lichtzeit. Z. vergl. Physiol., *37:* 107–117.
49. TRIBUKAIT, B. 1956. Die Aktivitaetsperiodik der weissen Maus im Kunsttag von 16 bis 29 Stunden Laenge. Z. verg. Physiol., *38:* 479–490.
50. REMMERT, H. 1955. Untersuchungen ueber das tageszeitlich gebundene Schluepfen von Pseudosmittia arenaria. Z. vergl. Physiol., *37:* 338–354.
51. STOPPEL, R. 1910. Ueber den Einfluss des Lichtes auf das Oeffnen und Schliessen einiger Blueten. Z. f. Bot., *2:* 369–453.
52. UEBELMESSER, E. R. 1954. Ueber den endonomen Tagesrhythmus der Sporangientraegerbildung von *Pilobolus.* Arch. Mikrobiol., *20:* 1–33.
53. LORENZEN, H. 1957. Synchrone Zellteilung von *Chlorella* bei verschiedenem Licht-Dunkel-Wechsel. Flora, *144:* 473–496.
54. HASTINGS, J. W., and B. M. SWEENEY. 1959. The *Gonyaulax* clock. Pp. 567–584. *Photoperiodism and Related Phenomena in Plants and Animals,* ed. Withrow. Washington: A.A.A.S.
55. BOUGHTON, D. C., F. O. ATCHLEY, and L. C. ESKRIDGE. 1935. Experimental modification of the diurnal oocyst-production of the sparrow coccidium. J. Exper. Zool., *70:* 55–74.
56. BOYD, G. H. 1929. Experimental modification of the reproductive activity of *Plasmodium cathemerium* J. Exper. Zool., *54:* 111–126.
57. EGAMI, N. 1954. Effect of artificial photoperiodicity on time of oviposition in the fish Oryzias latpes. Annot. Zool. Jap., *27:* 57–62
58. MILLER, R. S. 1955. Activity rhythms in the wood mouse, *Apodemus sylvaticus,* and the bank vole, *Clethryonomys glareolus.* Proc. Zool. Soc. London, *125:* 505–519.
59. PIRSON, A., and H. LORENZEN. 1958. Ein endogener Zeitfaktor bei der Teilung von *Chlorella.* Z. f. Bot., *46:* 53–66.
60. ASCHOFF, J., and K. HONMA. 1959. Art- und Individualmuster der Tagesperiodik. Z. vergl. Physiol., *42:* 383–392.
61. CROWCROFT, P. 1954. The daily cycle of activity in British shrews. Proc. Zool. Soc. London, *123:* 715–729.
62. DUNNETT, G. E., and R. A. HINDE. 1953. The winter roosting and awakening behaviour of captive great tits. Brit. J. Animal. Beh., *1:* 91–95.
63. SCHEER, G. 1952. Beobachtungen und Untersuchungen ueber die Abhaengigkeit des Fruehgesanges

der Voegel von inneren und aeusseren Faktoren. Biol. Abhandl., Heft *3/4:* 1–68.

DISCUSSION

BÜNNING: It is certainly interesting from an ecological standpoint to know in which way the length of the periods is depending on continuous white light or on continuous darkness. But for the physiological analysis it is perhaps more important to know that continuous light has quite different effects, depending on the light quality. In *Phaseolus* and in several other plants the periods in continuous red light are by 2–5 hours longer than in continuous darkness, whereas in continuous far-red light these are shorter than in darkness. White light, thus, means in certain cases an interaction of 2 antagonistic effects.

ASCHOFF: I agree fully that different wave-lengths will have different influences on the clock-system; also it may be more important to study quality of light instead of intensity. On the other hand, as I in my talk was mostly concerned with features of the free running oscillation and with its entrainment by Zeitgeber, it seemed reasonable to me to refer mainly to all the several experiments with different intensities of white light. Under natural conditions, white light is the main Zeitgeber, and one can use it to study general clock-mechanisms.

CLOUDSLEY-THOMPSON: If the change in phase-length depending on light intensity has an adaptive function in altering the time of the onset of activity with changing season, it would seem that the clock may not only be regulated in this way, but may also be synchronized by a Zeitgeber each day. My work on terrestrial anthropods suggests that under one set of constant condition the free-running period may be almost exactly 24 hours, yet it may be less or more than this under another. Why, therefore, do you say that an exact 24-hour period is unlikely to be found? In your figures of the times of the onset of activity in the finch, the curve slopes upwards or downwards according to the light intensity. Surely, if the correct intermediate intensity was selected, it would be horizontal.

ASCHOFF: Full agreement, that by using the right constant conditions the spontaneous period may be 24 hours. This never has been refuted.

ENRIGHT: Dr. Aschoff has, in his paper, indicated that he is not seriously concerned with amplitude in activity cycles; but yet he has emphasized what he has called "α" and "ρ" i.e., the proportion of the cycle during which the organism is active above some zero level. I would like to suggest that α-ρ measurements may, in fact, be only a reflection of amplitude, no less, but no more reliable, than amplitude as a characteristic of a given rhythm.

My own work on activity rhythms has produced, under some conditions, a discontinuous, peaked graph such as shown in Dr. Aschoff's graphs for finches, for example. By changing experimental conditions—not with regard to temperature or light intensity, but, for example, simply by depriving the animals of a sand substrate, a factor which would presumably have no bearing on the "rate of energy flow through the system"—the activity data can be transformed to a continuous function with, in fact, no zero level.

I would suggest, then, that light, or substrate, or the like, can markedly affect amplitude of an activity rhythm by determining the zero, or threshold level, below which cyclic processes continue but are not evidenced by overt activity; that the position of this threshold level may be shifted upward or downward by a whole array of factors from both the recent past history of the organism, and the experimental conditions; and that this shifting of threshold would also result in changed values of α and ρ. If the threshold is shifted upward enough, there will be zero amplitude, and zero α; shifting downward enough there will be continuous activity and a zero ρ.

I bring this point up not to discount the significance of α and ρ as characteristics of a given cycle, but rather to emphasize what I believe is the very real importance of amplitude measurements of the cycles; to suggest that α and ρ may simply be different aspects of the amplitude of a truncated continuous cycle.

ASCHOFF: I agree that α and ρ are also measurements of the amplitude so long as the period is constant. If, on the other hand, amplitude remains constant, the $\alpha = \rho$ ratio is only a function of the average level. The threshold above and below which we measure activity-time and rest-time is quite clearly arbitrary. This threshold may be zero as well as any other value of the ordinate on which we draw the observed activity; as well it may be the surface of a layer of sand wherein the organism vanishes during rest-time and where from it arises with beginning activity-time. The main idea is not to shift the threshold—which clearly would change the $\alpha = \rho$ ratio—but to keep the threshold at a definite value of the ordinate. If then $\alpha = \rho$ changes without a change in amplitude (upper + lower width), the level must have been changed. The whole problem is easy to understand if one draws up a curve of body temperature and uses 37° as a threshold. The upward step of the level by one degree during fever (without change in amplitude, as is often the case) will bring an increase in $\alpha:\rho$ ratio.

KALMUS: *LD* means lethal dose in toxicology and is the measurement of insecticidal action. One might perhaps avoid such abbreviations like *LD* 50 per cent in describing charges of illumination.

ASCHOFF: *L* and *D* for light and dark are now so commonly used that it seems rather difficult to avoid using them.

KALMUS: Deviation from an exact 24-hour period in constant conditions should not be used as the sole

criterion for endogenouity. Continuation of an inverted or phase-shifted rhythm in constant conditions is another important criterion.

ASCHOFF: Exactly these types of experiments have been used to make proof of an exogenous periodicity. Hypothetical explanations for the continuation of inverted rhythms as exogenous ones are, for instance, given by F. A. Brown. Serious explanations may be based on the possibility of semistable phase control. To avoid such criticisms, only the spontaneous period deviating from 24 hours should be taken as proof for endogenous.

RAWSON: What evidence is there to substantiate the concept of an *angular velocity* within a one-day period? It appears to me that most of our evidence is based on the measurement of *one event per day* such as peak of activity or onset of activity but that we do not have many time measures by organisms throughout the day. There are a few measures of time within the day, such as those of sun compass orientation, where the "clock" is read during the day. Until this kind of evidence is analyzed we can say nothing about *angular velocity* of the rhythm or even whether the expression "angular velocity" is a useful term describing the circadian rhythm of an organism.

ASCHOFF: The statements with regard to angular velocity have been started only from arguments which are implicit in an oscillating system. The first question was whether one can assume or not that the varying speed of the system is correlated to *LD* or activity- and rest-time respectively. The next question is by what means we perhaps can measure the speed; few possibilities are discussed in the paper.

SOLLBERGER: I want to point out that the amplitude may often rise with the level, just indicating an increased load on a constantly running system. In that case only a change in the relation between amplitude and level ("amplitude line") signifies a real change in "angular velocity." Perhaps the latter may preferably be called average velocity instead.