AN EXOGENOUS REFERENCE–CLOCK FOR PERSISTENT, TEMPERATURE–INDEPENDENT, LABILE, BIOLOGICAL RHYTHMS [1,2]

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The phenomenon of persistent rhythmicity of one or more of their vital processes is widespread among animals and plants. By persistent rhythmicity is meant that the rhythm still continues when conditions are held constant with respect to all factors generally conceded to influence the organisms.

Reviews of this subject have included those by Bünning (1936, 1956a, 1956b), Jores (1937), Kalmus (1938), Welsh (1938), Park (1940), Kleitman (1949), Calhoun (1944, 1945–46), Korringa (1947), Webb (1950), Caspers (1951), Cloudsley-Thompson (1953), Bruce and Pittendrigh (1957), and Brown (1957d, 1958). The broad distribution of such rhythmicity is suggestive of an hypothesis that all living things have potentially the means of persistent rhythmicity provided it has a period close to that of one of the natural geophysical rhythms. The organismic rhythms usually are essentially temperature-independent in their frequencies, whether the periods are solar-daily, lunar or annual.

Most of the observed rhythms are clearly endogenous, and are labilely adaptable in form and phase relationships to the needs of the organism. Much has been learned, particularly in recent years, as to the properties, including modifiability, of this endogenous rhythmicity. The fundamental problem, however, that of the timing mechanism of the rhythmic periods, has largely eluded any eminently reasonable hypotheses in terms of cell physiology or biochemistry. The problem was already a difficult one when only solar-daily cyclicity was under consideration, but especially in recent years it has been found that one and the same organism may simultaneously possess overt daily and lunar tidal cycles of two bodily processes. Further, the possession of persistent lunar monthly (Brown, Bennett and Webb, 1958) and even annual cycles (Bünning and Müssle, 1951; Bünning and Bauer, 1952; Brown, 1957c) in constant conditions has emphasized the magnitude and complexity of this basic problem.

Added to the property, temperature-independence, in indicating the unconventional character of the rhythm-timing mechanisms, are the repeated demonstrations of the immunity of the frequency-determining mechanism to most metabolic poisons.

Recently, evidence has been rapidly accumulating pointing to the possession by living organisms of basic metabolic cycles of the natural geophysical frequencies,

produced in the organism by an external cyclic stimulus still operative in so-called laboratory constant conditions (Brown, 1957; Brown, Shriner and Webb, 1957; and Brown, Webb and Macey, 1957). These cycles are not phase- or form-labile. The problem of a common explanation for persistent rhythmicities of all the well-known natural frequencies including the year becomes at once more susceptible to reasonable working hypotheses as to their mechanism when it is firmly established that protoplasm in "constant conditions" is, fundamentally, exogenously rhythmic.

For the study to be reported here, the potato and carrot were selected as organisms neither of which appears to possess any obscuring, labile, endogenous rhythms. It was considered that such organisms would reveal most readily any extant basic protoplasmic cyclicities and also permit easier analysis of any mechanisms they involved.

On the basis of this hypothesis, of an exogenous reference clock providing the timing of cyclic periods, the often-described endogenous rhythms would be considered a consequence of the evolution by the organism of adaptive labile cyclic changes, utilizing the basic exogenous cycle-timing mechanism. The endogenous mechanisms could be inherited. The only inherited aspect of the exogenous cyclicity would be the fundamental protoplasmic responsive systems which are involved.

MATERIALS AND METHODS

The potatoes, *Solanum tuberosum,* were of the Idaho variety and were purchased from local grocery stores. The carrots, *Daucus carota,* were similarly purchased from local stores. Using a cork-borer, small cylinders, 2.2 cm. in diameter and about 1.5 cm. tall, were prepared from the potatoes in such a manner that each carried an eye on the center of its upper surface. These were permitted to heal their cut surfaces before being set, in shallow water, in respirometer vessels where the same individual organisms were retained up to three or more months. These always gave rise to sprouts and usually also to a root system, and in some instances even developed new tubers up to a centimeter or more in diameter during their sojourn in the respirometers. For the carrots, short cylindrical sections, about the size of the potato-cylinders, were cut and allowed to heal over before being placed in respirometers.

The respirometers have been described earlier. These were originally designed by Brown (1954) and later modified (Brown, 1957a) to permit maintenance of constant pressure.

Five independent barostat-respirometer ensembles, each with 4 respirometers recording as a unit, were in nearly continuous operation during the period of study, Feb. 1, 1956 through Feb. 28, 1958. The potatoes in the respirometers were in constant illumination (estimated at 0.05 ft. c. at the site of the plants) from incandescent lamps supplied by a voltage-regulated line. The temperature, 20° C., was maintained constant by the respirometers being immersed in a large non-stirred, copper water-bath (the barostat) deeply immersed in an outer, stirred, steel (55-gal. drum) water bath, with the latter cycling with a few-minute period within a ± 0.05° C. range. The pressure was kept constant, 28.5 in. Hg, through hermetically sealing the respirometer-recorder-containing barostat and then aspirating the system to this level. Oxygen and CO_2 tensions were maintained essentially constant through

use of the principle of continuous O_2-replacement together with CO_2 absorbents, and there were clearly no regularly cyclic fluctuations in these substances. Also, the sealed, water-included systems allowed for no changes in humidity.

With a single exception (12 days) the copper tanks, or barostats, remained sealed for periods ranging from 2 to 8 days, with an average of 4.46 days. At these intervals the organisms were exposed for 15–20 minutes to laboratory conditions which were relatively constant over the year. No work was done within 15 feet of outside windows; the laboratory fluorescent illumination at table top was about 45 ft. c. (The carrot study was carried out wholly in a dark-room without any natural illumination.) The room temperature was relatively constant, about 75° F., except for slightly higher values during the summer months. The barostats were opened at various hours of the day from 8 AM to 10 PM. Excluding those days the respirometers were opened to renew the O_2 and the CO_2 absorbent, a total of 2485 uninterrupted calendar days of data were obtained.

The recording systems of the respirometers possessed two points of slight mechanical frictional resistance, a) a two-point pivotal, spring-scale bearing, and b) the point of contact of the ink-writing pen with the slowly moving paper. These resulted in random, spurious apparent intra-hour fluctuations in rate of O_2-consumption. Since the principle of operation of the recorder was one with which the hourly values of O_2-consumption were obtained by calculating the differences between consecutive hourly markers on a continuing trend-line denoting cumulative O_2-consumption, these spurious fluctuations in apparent rate could, and undoubtedly did, produce larger hour-to-hour differences than bore any significance. Hence, time units of less than three hours (three-hour "moving means") were never used in determining the mean rates centered on any given hour. By this means the random mechanically induced error was reduced to about one-third its single-hour influence. For most of the study reported here, a weighted ($1:2:3:3:3:2:1$) seven-hour "moving mean" was used. This reduced by essentially 90% the random fluctuations while retaining all the precision of measurement of average, actual, O_2-consumption for this longer interval, as modified by its weighted character. The shorter period, three-hour, means were found necessary, however, to expose the relationship between day-by-day 6 AM deviations in O_2-consumption from daily linear trends and the concurrent day-by-day mean rates of barometric pressure change for the 2–6 AM interval. Although some clearly significant short-period fluctuations were obscured, therefore, by the seven-hour weighted "moving means," these were considered superior to the shorter periods for the accurate description of the general characters of the longer-period, daily and annual cycles to be described herein.

The records for the five completely independent, respirometer-recording systems were first dealt with individually and three-hour and weighted seven-hour "moving means" were prepared month by month for the period of study. From the latter values were calculated the mean daily rates of O_2-consumption and the data were then converted into hourly deviations from the solar daily means. The number of uninterrupted days of data from the 24 months of study ranged from 93 to 129 each month. The hourly deviations for all the respirometers operating were averaged for each calendar day, and these average daily cycles then converted to hourly deviations from a 1 AM to 12 midnight linear trend-line. This will be referred to as the deviations from linear daily trend. From these data the forms of the mean

daily cycles for each month were obtained. The slope of this linear trend-line it-self shows apparent monthly and annual periodisms which have been treated else-where (Brown, 1957c; Brown, Bennett and Webb, 1958). The trend involved a mean daily increase during the two-year study of 6.7%, and included, as a large
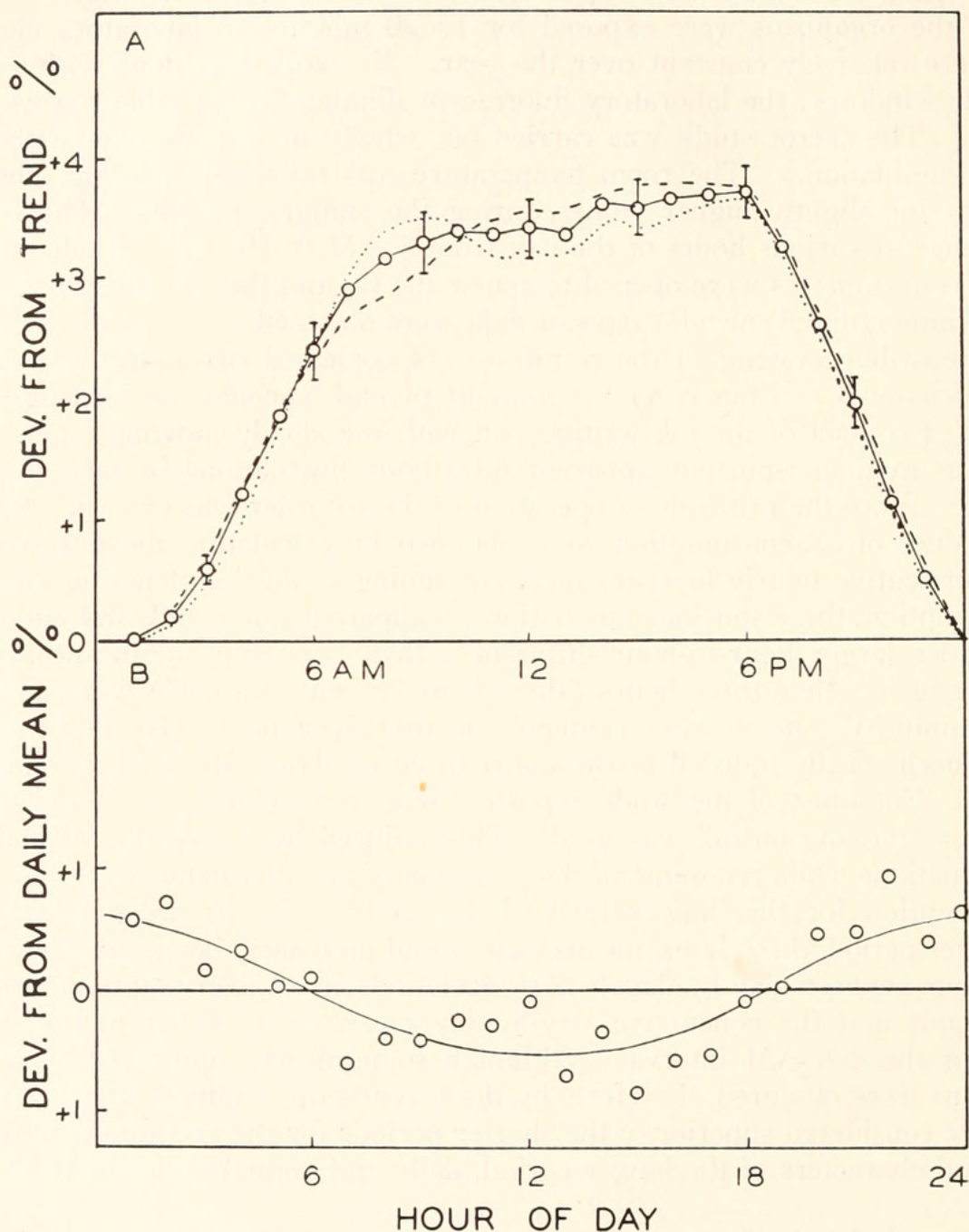

FIGURE 1. A. The mean solar-day cycle of O_2-consumption in the potato (solid line) with standard errors for selected hours. This is expressed as % deviations from linear daily trend. The dashed curve is the cycle for the first year of study, the dotted curve, for the second. B. The mean apparent sidereal-day cycle of the potato for the two-year period of study.

component, the apparent smoothly gradual recovery over a 3- to 5-day period, from the inhibitory influence of the room-illumination intensity. The mechanical re-cording system, itself, departed from linearity over its total range by 10%, departing in such a direction that there would be expected on this basis an average of about 2% increase per day.

An entirely independent and parallel study was made of O_2-consumption of the sections of the carrots, for the 8-month period Oct. 1, 1956 through May 31, 1957. Two respirometer-barostat ensembles were employed for the first three months, and four for the remaining five months. These were maintained in darkness in a photographic darkroom about 60 feet away from the place of the potato study, but similarly on the ground floor of Cresap Biological Laboratory, a three-story steel and mortar building. The respirometers were maintained and the data processed by a person not involved until the termination of the carrot study in the paralleling and continuing potato study.
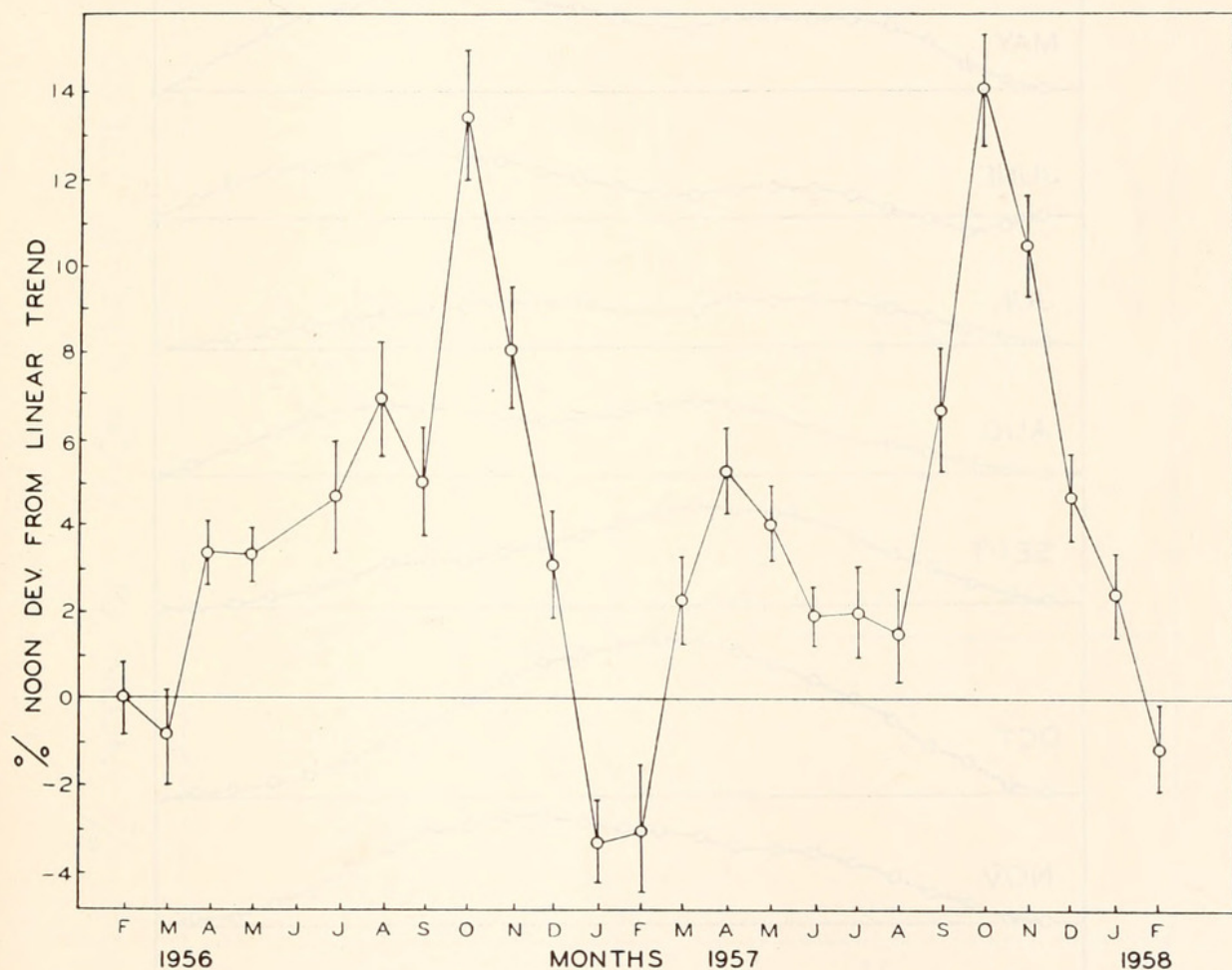

FIGURE 2. The relationship between average % noon deviation in O_2-consumption in the potato from linear daily trend and month of year during the 25-month study. Standard errors of means are shown.

RESULTS

During the period, Feb. 1, 1956 through Feb. 28, 1958, in the study of the potato, the only days omitted were May 25, the month of June and the first three days of July, 1956, and October 4, 1957.

The form of the mean daily deviation from trend, expressed as percentage of the daily mean rate, is shown, with the standard errors of arbitrarily selected mean values, in the solid-line curve of Figure 1, A. The errors of the other values are quite comparable in size. Superimposed on this are the mean cycles for each of the two years separately: Feb. 1, 1956 through Jan. 31, 1957 (the dashed curve) and
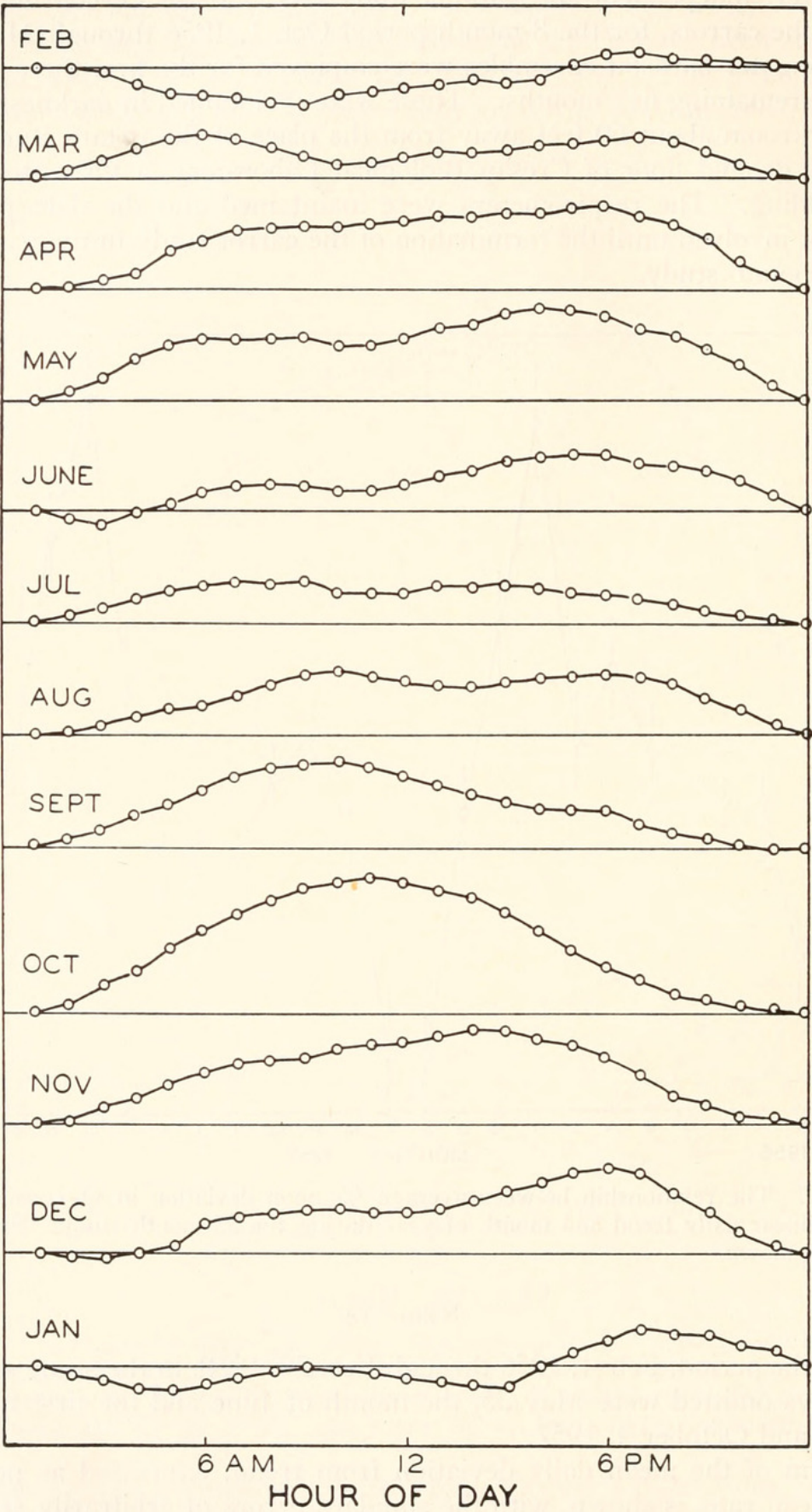
FIGURE 3. The forms of the average daily cycles for each month of the year obtained in the two-year study. The ordinate values are deviations in comparable arbitrary units.

Feb. 1, 1957 through Jan. 31, 1958 (the dotted curve). The average amplitude of the daily cycle was clearly quite reproducible for the two years at about 3.7%. There was also clear suggestion, in the skewed cycle form, of a bimodality with morning and afternoon maxima, a condition more conspicuous for the second than for the first year of study. The mean sidereal-day cycle (23 hours, 56.07 minutes) for the two-year period is shown in Figure 1, B. This was obtained by displacing
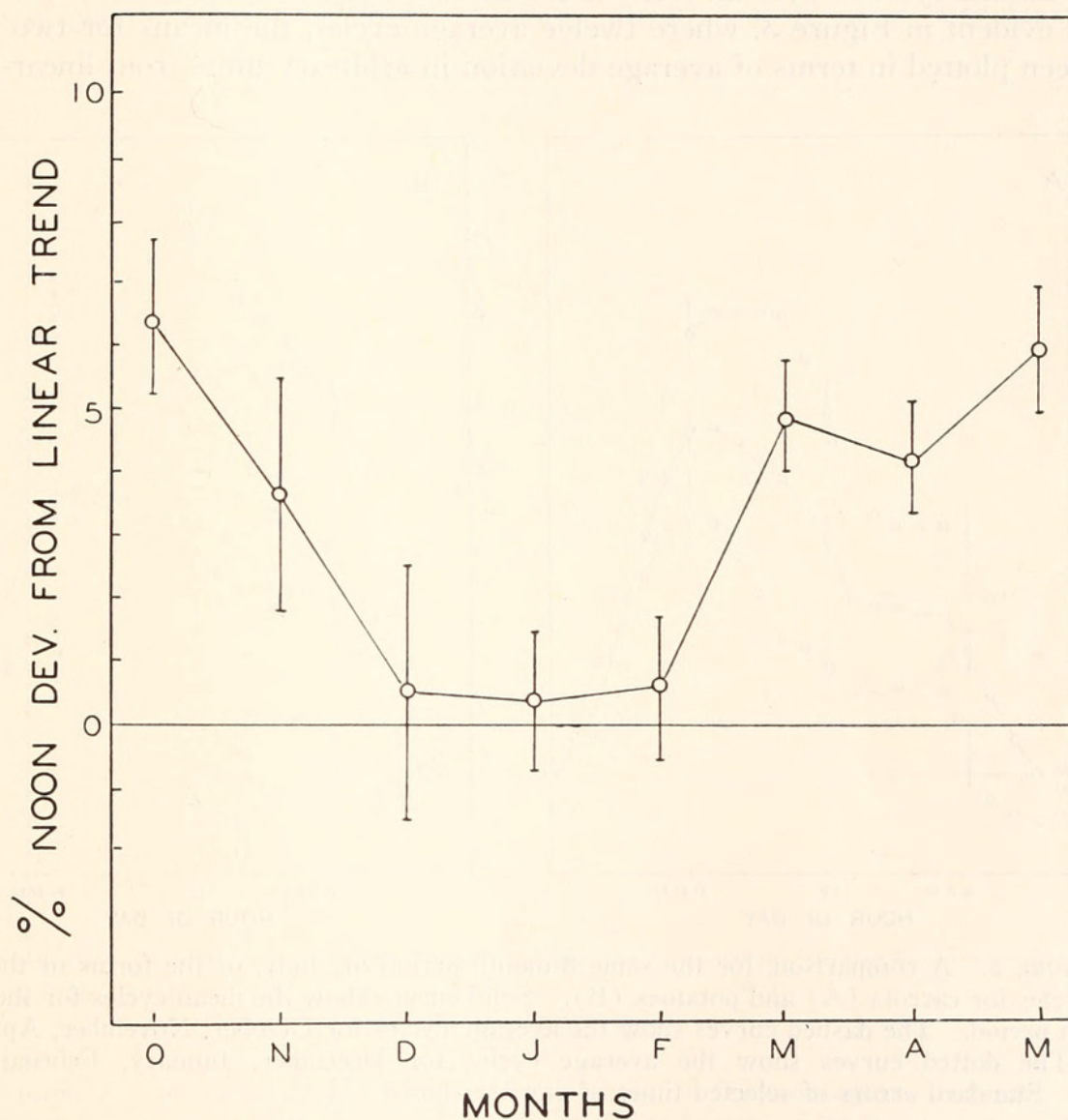

FIGURE 4. The relationship between the noon deviations in O_2-consumption from linear daily trend and calendar month in the *carrot* during an 8-month study. The standard errors are depicted.

the consecutive mean monthly solar-day cycles each by two hours to the right during the two-year period, to bring into reasonably close synchrony (± 1 hour) the hours of the sidereal day. The numbered hours are fixed by the solar-day hours of the first month, February, 1956. This process also randomizes daily trend.

The form and amplitude of the solar daily cycle showed differences from month to month which revealed that it was undergoing a modulation of an annual frequency. This was quite evident when one used, for example, the parameter of average monthly noon deviation, in percentage, from 1 AM to midnight daily

linear trend. The deviations, month-by-month, for the period Feb. 1, 1956 through Feb. 28, 1958, together with their standard errors, are depicted in Figure 2. These indicate minimum annual values, involving often even apparent cycle inversion, during the coldest months of the year and a major maximum in the month of October. A lesser, or incipient, maximum occurred in April–May. The maximum range is seen to extend from − 3.4% to + 14.2%.

An annual cycle in over-all form of the mean daily cycles for the months of the year is evident in Figure 3, where twelve average cycles, the means for two years, have been plotted in terms of average deviation in arbitrary units from linear-trend.
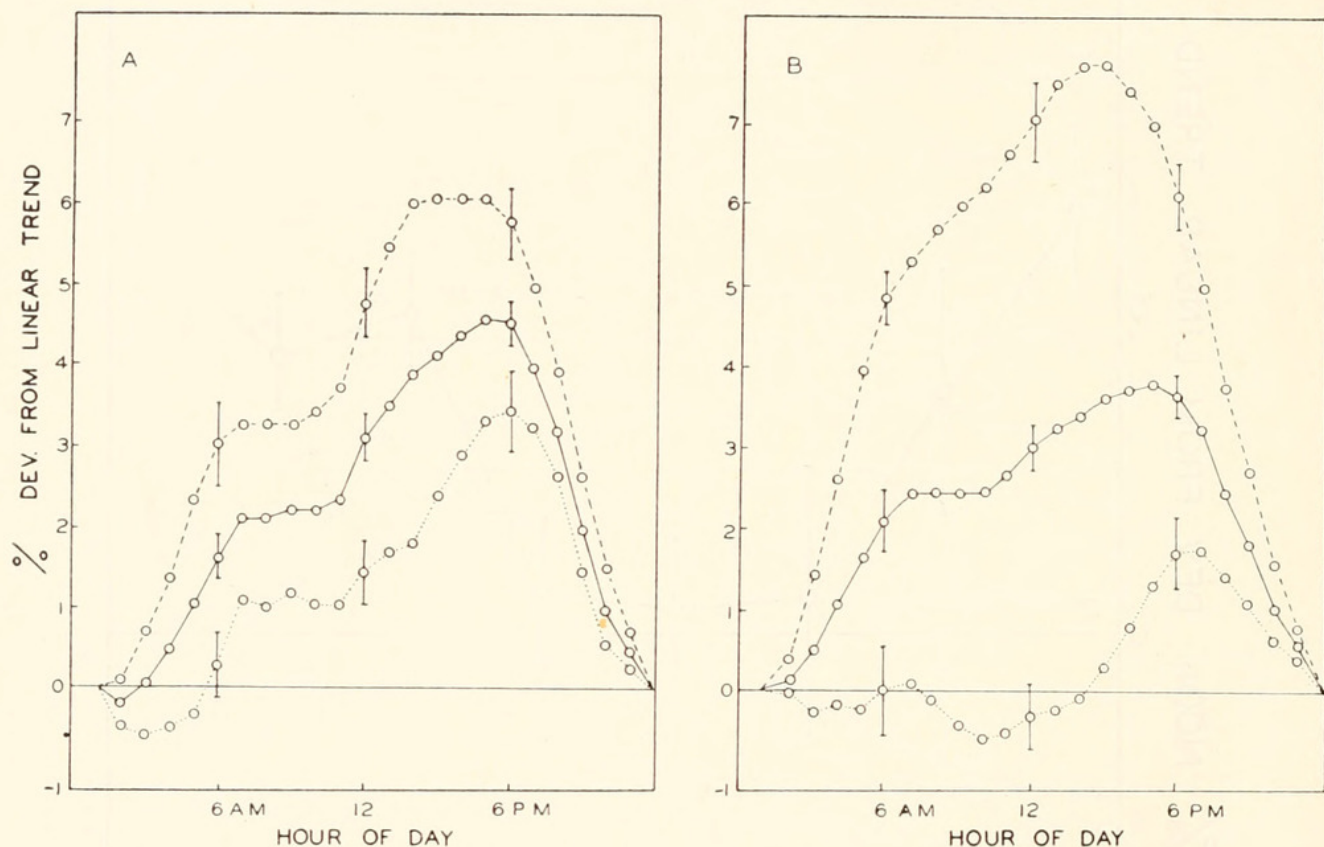

FIGURE 5. A comparison, for the same 8-month period of study, of the forms of the mean daily cycles for carrots (A) and potatoes (B). Solid curves show the mean cycles for the whole 8-month period. The dashed curves show the average cycles for October, November, April, and May. The dotted curves show the average cycles for December, January, February, and March. Standard errors of selected times of day are shown.

Although these data are not expressed as percentage deviations, they do illustrate the gradually-changing form of the cycles from unimodality with essential inversion in February, but with a 7 PM maximum, through a period of bimodality with the two daily maxima gradually converging towards noon to reach unimodality with a maximum at 11 AM in October. Thereafter, bimodality reappears and continues, becoming only feebly evident as an apparent residual in the essentially unimodal inverted cycle of January which like the succeeding month, February, has a 7 PM maximum.

The study of the carrot revealed striking similarity of its major mean cycles with those of the potato. Figure 4 shows the mean % noon deviation from linear trend for each of the eight months. Like the results obtained with the potato for

the same calendar period, this passed from an early-fall higher value, through a
winter minimum and back to a higher spring value.   Fewer data were available dur-
ing the first three months, hence the errors were larger.   The range was less than
for the potatoes.   Figure 5, A and B solid curves, compares the mean 8-month daily
cycles for the carrot and potato, and the average cycles for the two fall and two
spring months (dashed curves) as compared with those for the four intervening
colder months (dotted curves),   The similarities of these two widely different
kinds of plants and plant portions (roots vs. stems) for the same periods, in the %
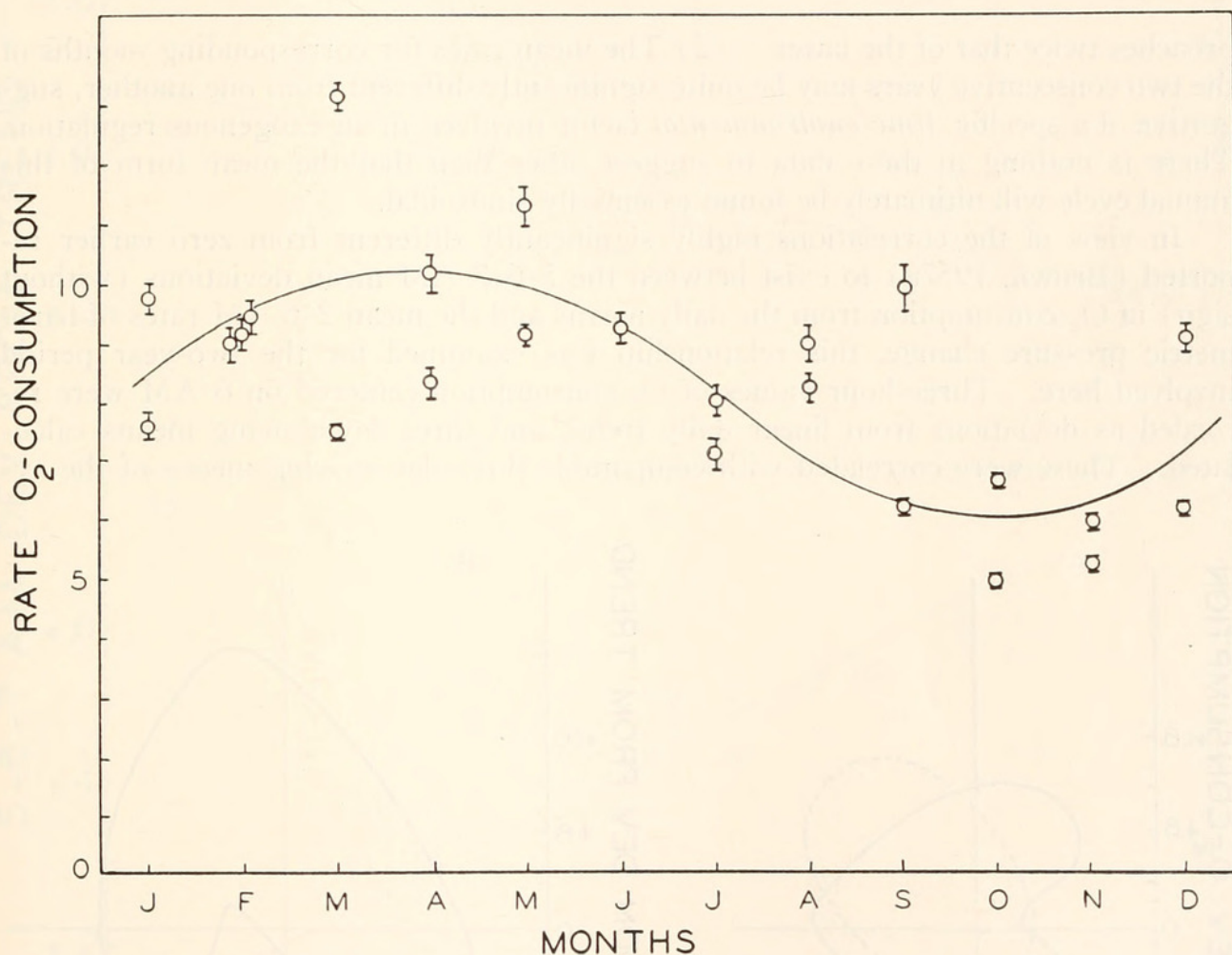

FIGURE 6.   The relationship between mean rates of O₂-consumption and each of the 24
months studied during 1956, 1957, and 1958, and time of year.   Standard errors of the means
are indicated.

amplitudes of the fluctuations, in the times and the changing times with time of year
of the primary maxima, and in the times of secondary, or incipient, maxima, are
strikingly apparent from the figures.

A second kind of annual cycle appears also present in the data.   This is in the
mean daily metabolic rates.   In Figure 6, are to be found the mean monthly rates
of O₂-consumption, in arbitrary units, for each of the 24 months of study, together
with their standard errors.   Two conclusions are evident from the figure: (1)
The maximum rate of O₂-consumption occurs in the April–May period of the year
and minimum rate in October–November.   The rate for the former period ap-

TABLE I

Signs of the average monthly correlations of the 6 AM deviations from linear
trend with the mean 2–6 AM rate of barometric pressure change

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Year 1		−	−	+			+	+	+	+	−	−
Year 2	−	−	+	+	+	+	+	+	+	+	−	−
Year 3	−											

proaches twice that of the latter. (2) The mean rates for corresponding months of the two consecutive years may be quite significantly different from one another, suggestive of a specific, *time-environmental* factor involved in an exogenous regulation. There is nothing in these data to suggest other than that the mean form of this annual cycle will ultimately be found essentially sinusoidal.

In view of the correlations highly significantly different from zero earlier reported (Brown, 1957a) to exist between the 5–6–7 AM mean deviations (without sign) in O_2-consumption from the daily means and the mean 2–6 AM rates of barometric pressure change, this relationship was examined for the two-year period involved here. Three-hour values of O_2-consumption centered on 6 AM were recorded as deviations from linear daily trend, and three-day moving means calculated. These were correlated with comparable three-day moving means of the av-
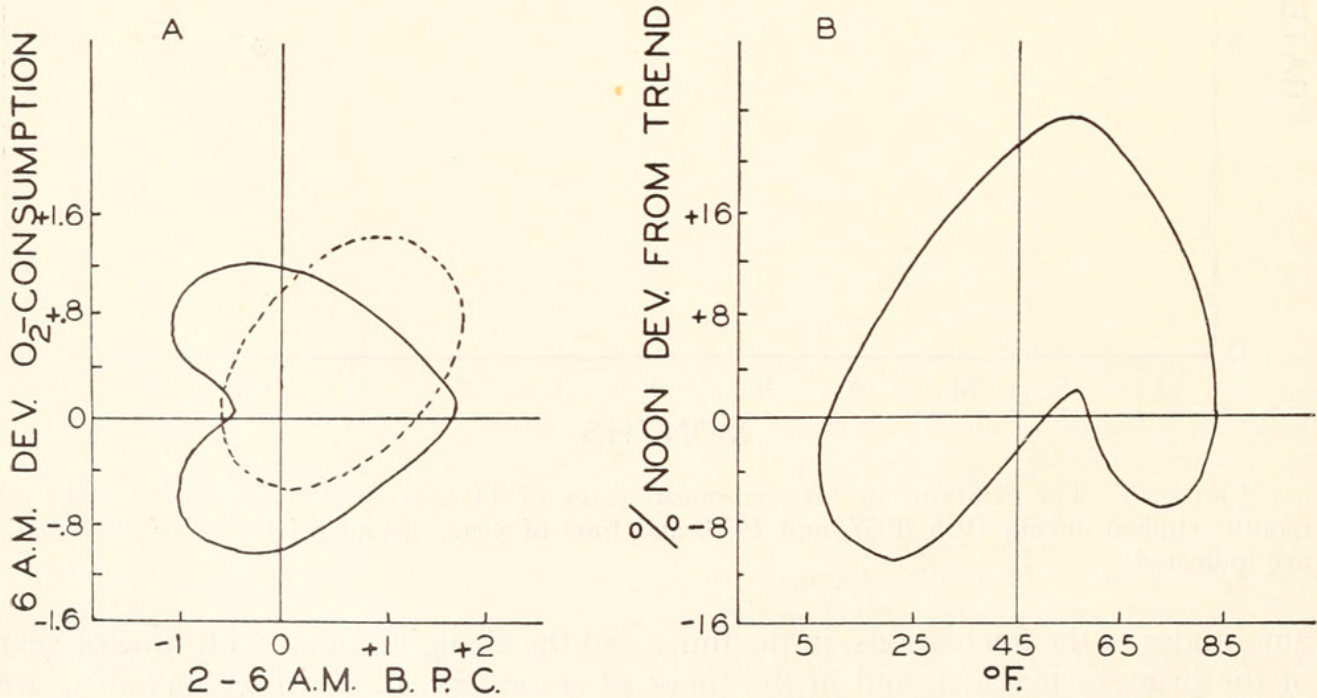

FIGURE 7. A. Solid line: An outline of the general form of the scatterplot between mean 2–6 AM rate of barometric pressure change and the 5–6–7 AM mean deviation of rate O_2-consumption from daily trend for the same day during the "colder" months (see text). Broken line: The same for the "warmer" months. Data for both involve three-day moving means. The two patterns together include 98% of all points. B. An outline of the form of the scatterplot (97% of all points) between % noon deviations from linear trend in potatoes in constant conditions and concurrent outdoor air temperature, taken from data of 149 non-overlapping three-day averages.

erage 2–6 AM rate of barometric pressure change, for the corresponding days. It should be emphasized that only a single value was used for each day for each phenomenon; hence, this did not involve a correlation of parallel daily cyclic trends. A positive coefficient, highly significantly different from zero, was obtained. This correlation, as one would anticipate in view of the essentially aperiodic, large climatic barometric pressure changes, rapidly drops to insignificance as one correlates
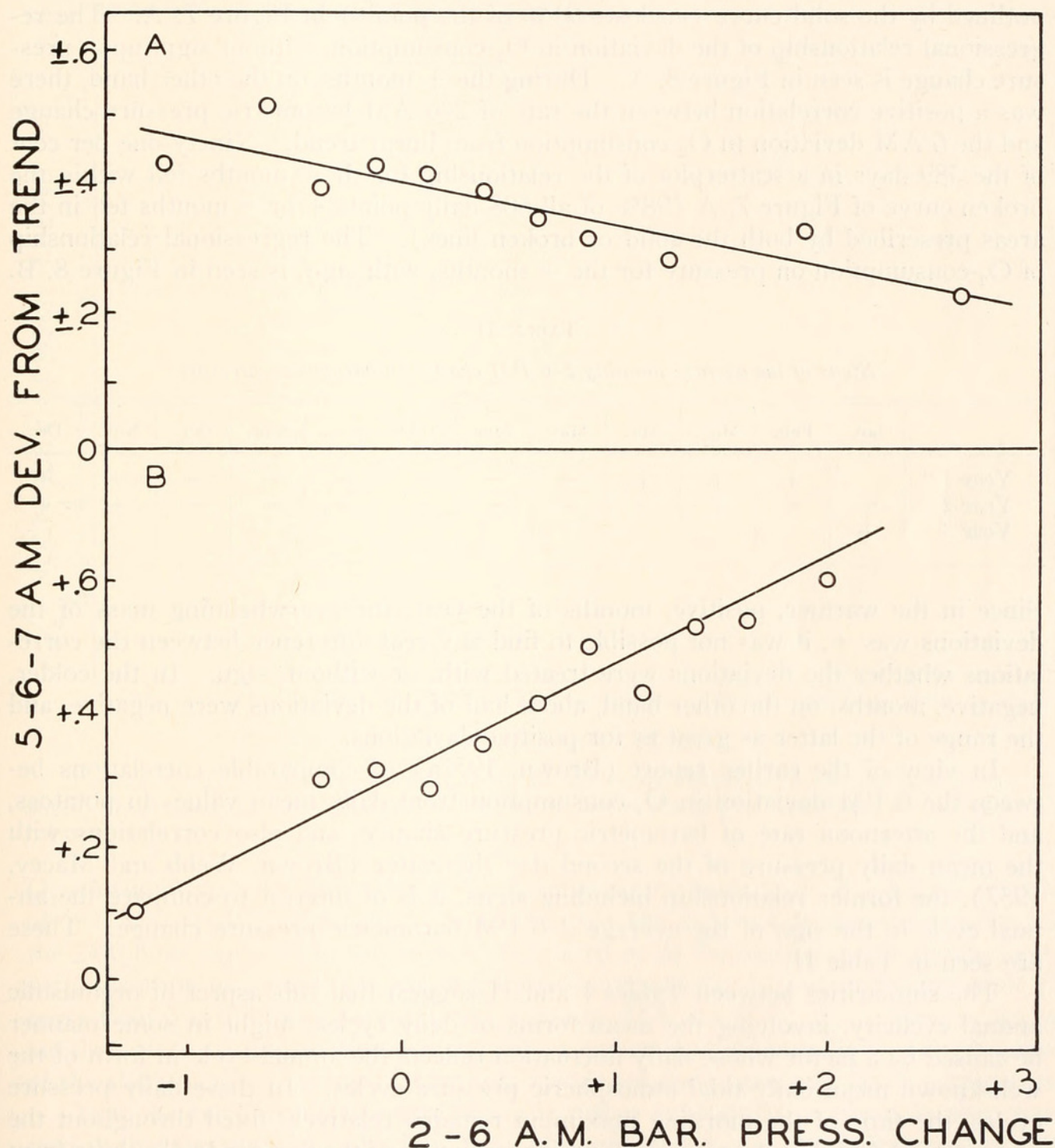

FIGURE 8. The regressional relationship of the average three-hour rate of O_2-consumption of the potato centered on 6 AM, and expressed as deviation from linear daily trend, on the average rate of barometric pressure change during the 2–6 AM interval for the same morning for the colder months of the year (see Table I). $P < 0.005$. B. The relationship comparable to that in A, but for the warmer months of the year (see Table I). $P < 0.001$.

in increasing lag on lead relationships up to two to three days (Brown, 1957a) indicating a direct response of the organisms to some pressure-change-correlated external variable. But this relationship was found to contain a characteristic sign-change twice a year as seen in Table I. In this table, a dash indicates those months in which there was a negative correlation between the rate of the 2–6 AM barometric pressure change and the 6 AM deviation, *without sign,* from linear trend. The form of the scatterplot relationship for the 299 days of this negative period is outlined by the solid curve (encloses 91% of the points) in Figure 7, A. The regressional relationship of the deviation in O_2-consumption, without sign, upon pressure change is seen in Figure 8, A. During the + months, on the other hand, there was a positive correlation between the rate of 2–6 AM barometric pressure change and the 6 AM deviation in O_2-consumption from linear trend. Ninety-one per cent of the 389 days in a scatterplot of the relationship for these months fell within the broken curve of Figure 7, A (98% of all 688 daily points + or − months fell in the areas prescribed by both the solid or broken lines). The regressional relationship of O_2-consumption on pressure for the + months, with sign, is seen in Figure 8, B.

TABLE II

Signs of the average monthly 2–6 PM change in barometric pressure

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Year 1		+	+	+	−	−	−	−	−	−	+	+
Year 2	+	−	+	−	−	−	−	−	−	−	+	+
Year 3	+											

Since in the warmer, positive, months of the year, the overwhelming mass of the deviations was +, it was not possible to find any real difference between the correlations whether the deviations were treated with, or without, sign. In the colder, negative, months, on the other hand, about half of the deviations were negative, and the range of the latter as great as for positive deviations.

In view of the earlier report (Brown, 1957a) of comparable correlations between the 6 PM deviation in O_2-consumption from daily mean values in potatoes, and the afternoon rate of barometric pressure change, and also correlations with the mean daily pressure of the second day thereafter (Brown, Webb and Macey, 1957), the former relationship including signs, it is of interest to compare the annual cycle in the sign of the average 2–6 PM barometric pressure change. These are seen in Table II.

The similarities between Tables I and II suggest that this aspect of organismic annual cyclicity, involving the mean forms of daily cycles, might in some manner be caused by a factor whose daily fluctuation reflects the annual cycle in form of the well-known mean daily tidal atmospheric pressure cycles. In these daily pressure cycles, the time of the morning maximum remains relatively fixed throughout the year at 9–10 AM, but the afternoon, major minimum of the day gradually shifts from about 2 PM in winter to about 7 PM in summer. This last is the basis for the sign changes in Table II. Thus, any pressure-correlated effective external physical factor could provide such an annual cycle in the daily cycles as that described herein.

Another clearly evident correlation is seen in the relationship of the % noon de-

viation from linear trend in the potato to the concurrent outside mean daily air temperatures.[3]   A two-year study of the comparison of non-overlapping three-day periods of air-temperature and of noon deviation of the daily cycles in constant conditions yields a scatter plot relationship as illustrated in Figure 7, B.   The line includes 97% of the 149 values.   The regressional relationship of noon-deviation of the potatoes on temperature (using 5° F. class intervals) is illustrated in Figure 9. The relationship seems adequately described as a linear one, but with a sign change near 57.5° F.   Calculation of the coefficient of correlation for noon deviation in
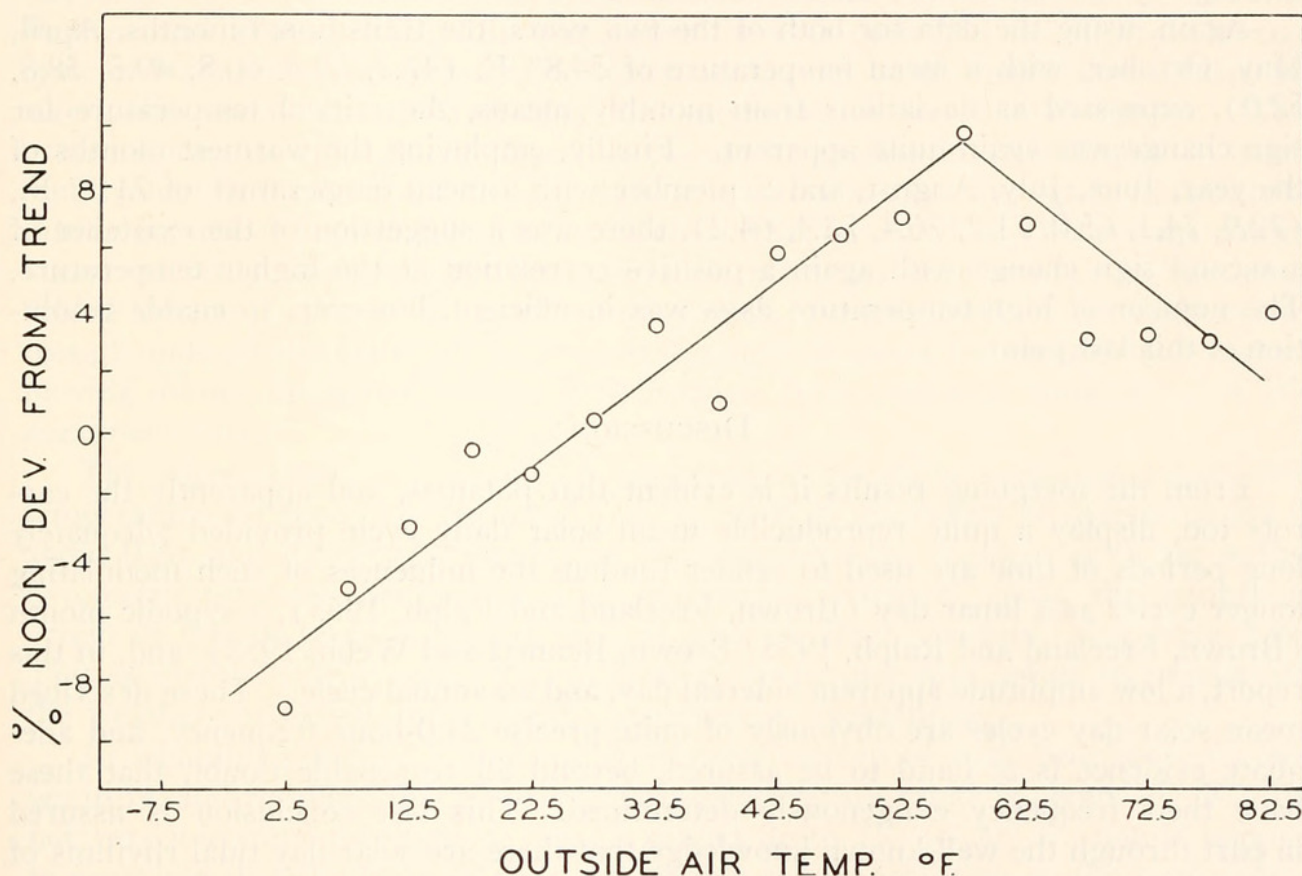

FIGURE 9.   The regressional relationship of the noon deviation in O_2-consumption in the potato, expressed as % deviation from daily linear trend on simultaneous outdoor air temperature.

O_2-consumption with the ± deviation in temperature from 57.5° F., yielded a value of − 0.51 ± 0.049.   This clearly indicates that the external factor responsible for the 24.0-hour cycles of metabolism is correlated in its fluctuations with air temperature, resulting in a condition where a spurious organismic Q_{10} of cycle amplitude of more than 3 could be apparent (e.g., in range 32.5° to 52.5° F.).   This provides another piece of information which will probably lead eventually to identification of the still unknown external factor responsible for the organismic basic periodisms.

That the relationship to temperature is rather substantial, is given further support in that the regressional relationship of noon deviation on temperature exhibited a sign change about 57° F. in the first year, 1956–57, just as it did again in the second, 1957–58, despite the fact that in 1956 there was no clear absolute summer de-

[3] These data were generously provided to me by the Chicago Office of the U. S. Weather Bureau.

cline in amplitude of the mean monthly cycles (Fig. 2) as was found in 1957. Also, during the winter months the correlations are observed (Fig. 9) to continue in the same linear relationship even at temperatures lower than the lowest mean monthly ones in the two years involved in this correlation (29.4°, 28.7°, 18.8°, 27.3°, and 26.3° F.) which averaged about 26° F. These last facts suggested intra-month significant temperature correlations which were borne out by investigation of the correlations using the data of the five coldest months now expressed as deviations from monthly means. The correlations continued in temperature ranges well exceeding any mean month-to-month difference.

Again, using the data for both of the two years, the transitional months, April, May, October, with a mean temperature of 54.8° F. (47.2, 60.5, 60.8, 49.5, 58.8, 52.0), expressed as deviations from monthly means, the critical temperature for sign change was again quite apparent. Finally, employing the warmest months of the year, June, July, August, and September with a mean temperature of 71.1° F., (72.9, 74.1, 65.0, 71.2, 76.4, 73.4, 64.2), there was a suggestion of the existence of a second sign change with again a positive correlation at the higher temperature. The number of high-temperature days was insufficient, however, to enable resolution of this last point.

DISCUSSION

From the foregoing results it is evident that potatoes, and apparently the carrots too, display a quite reproducible mean solar daily cycle provided adequately long periods of time are used to render random the influences of such modulating longer cycles as a lunar day (Brown, Freeland and Ralph, 1955), a synodic month (Brown, Freeland and Ralph, 1955; Brown, Bennett and Webb, 1958), and, in this report, a low amplitude apparent sidereal day, and an annual cycle. These described mean solar-day cycles are obviously of quite precise 24.0-hour frequency, and adequate evidence is at hand to be assured, beyond all reasonable doubt, that these have their frequency exogenously determined. This last conclusion is assured in part through the well-known knowledge that there are solar-day tidal rhythms of atmospheric pressure, together with the fact that the living organism has access to information of them through its responses to the day-to-day, essentially random, weather-induced, disturbances in their regularity. That the factor influencing the organism is not pressure itself, is evident from the fact that these and other experiments have involved organisms maintained for long periods in constant pressure. The external factor which is involved appears to have its primary action upon the organism at the times correlated with the early-morning rise in barometric pressure and the afternoon fall. These would presumably be the times of most rapid change in physical factors fluctuating with the day-night cycle, and hence be the times of their maximal stimulative effectiveness.

As pointed out earlier in this report, the presence of the well-known annual change in the form of the daily, tidal, barometric pressure cycles, and the described response of the organism in the late afternoon to a pressure-correlated external variable would have led to the prediction of the occurrence of an annual cycle in the form of the daily cycles. Such a prediction has been fulfilled in this study. This adds still further, therefore, to the assurance that the forms of the daily basic metabolic oscillations in living organisms are exogenous.

Since background radiation, too, possesses good mean solar-day cyclicity, and the organism follows the essentially random fluctuations in its cycle amplitudes from day-to-day (Brown, Shriner and Webb, 1957) very safely beyond what would be expected through chance, when and only when contemporary data are correlated, this constitutes a third line of evidence for exogenous origin of mean daily metabolic cycles.

The existence of an annual cycle in the potato in constant illumination, temperature, pressure, etc., was reported earlier for fluctuations in linear daily trend (Brown, 1957c), as were also synodic monthly cycles of this parameter (Brown, Bennett and Webb, 1958). In this paper there is described an apparent annual cycle in basic metabolic rate, a cycle which appears to be of simple sinusoidal character with maximum in April–May and minimum in October–November. This cycle involves an approximate doubling of rate in passing from minimum to maximum values in the annual cycle. Comparable, synodic monthly, cycles in metabolic rate in potatoes (Brown, Bennett and Webb, 1958) involved, as the average during a year of study, about a 15% increase from minimum (new moon) to maximum (third quarter) values. By further comparison, the amplitude of the daily cycles, though undoubtedly artificially depressed through the use of the seven-hour weighted moving means, displayed about a 3.7% increase from midnight minimum to 6 PM maximum values.

The regressional relationship of amplitude of the daily cycles on mean daily temperature for three-day periods (Fig. 9), with its coefficient of determination of about 0.26, and its critical temperature for sign-reversal, together with the earlier barometric-pressure-change reversing correlation, suggests again the exogenous origin of this daily cycle period, and, at least in large measure, also cycle form. This is especially true, since the relationship to temperature seems to persist into the weather-correlated, intra-month, temperature fluctuations.

In examining Figure 9 and noting the relationship of cycle amplitude to temperature, and recalling that the mean daily temperature range is about 16° F., with not very uncommonly single days with ranges up to 25° to 30° F., one is tempted to postulate that the factor that is responsible for transmitting to the organism in "constant conditions" information on outside air-temperature, is, through its temperature-correlated fluctuations alone, contributing importantly to the 24-hour periodic metabolic fluctuations themselves. In support of this hypothesis is the rough similarity in the average forms of the annual fluctuations in the amplitudes of ground-level daily temperature change and metabolic cycles. Both, as average for the two years, showed lowest values in the coldest winter months and highest values in late spring and late summer to early fall, with a summer amplitude reduction. The relationship between these two phenomena is seen in Figure 10. The October peak, so conspicuous for the metabolic cycles, is much less evident for the temperature changes.

For each year this relationship between these two phenomena appeared to trace out general ovoid form. The two-year mean month-by-month relationship is shown by the numerals 1 (January) through 12 (December), and the dotted ovoid curve roughly traces their course. It is interesting to speculate that this difference between the organismic and temperature annual cycles may find its explanation in the changing natural smog content of the atmosphere. The terpenes, volatilized from

plants, polymerized by the ultra violet light from sun, reach a maximum in October (personal communication from Professor F. W. Went). This smog may, through influencing the amount of heat absorption from sunlight, produce in October the highest amplitude daily temperature changes of the year at levels in the atmosphere where temperature changes produce greatest influence upon the factor directly affecting the organism. One process, known to be temperature-dependent, is the rate of spontaneous decay of cosmic-ray-derived mesons. The larger the atmospheric depth involved in the temperature change, in this instance, the larger would be expected the temperature influence.
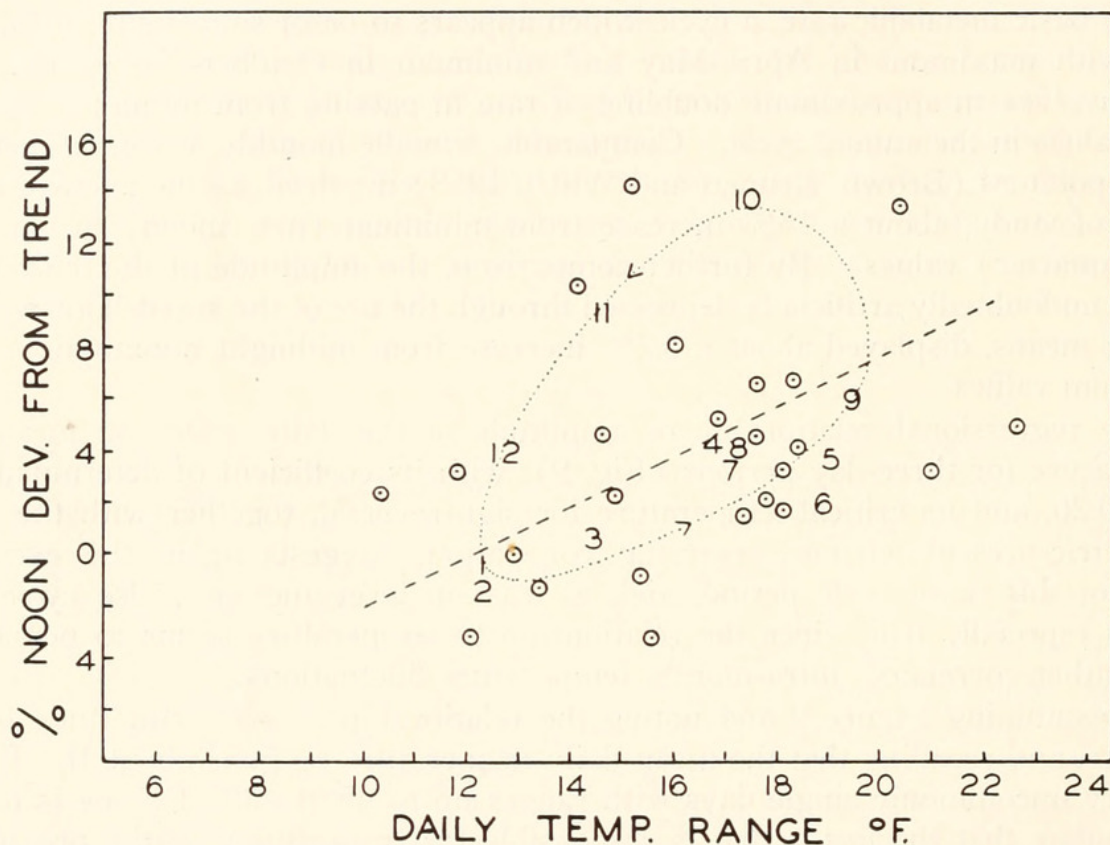

FIGURE 10. The relationship between mean monthly daily temperature ranges and the mean monthly noon deviations in O_2-consumption from daily trend. The Arabic numerals indicate the mean monthly relationships for both of the years, with January as 1, February as 2, March as 3, etc.

Now that it appears true beyond reasonable doubt that the living organism, as part of its cyclic geophysical environment, is exhibiting metabolic cycles with the natural solar day, lunar, and annual periods, the question arises as to what the relationship of these metabolic cycles might be to the well-known endogenous physiological and behavior cycles which have been abundantly described, especially during the past fifty years for species representing the gamut of the animal and plant kingdoms. Those studied experimentally have included especially the sleep-movements of plants, the spontaneous motor activity of numerous animals, physical and chemical adaptation of compound eyes, integumentary color changes, eclosion in flies, learned periodic behavior in insects and lunar-tidal rhythms of color-change and of motor activity. These cycles are clearly endogenous in most instances since the forms and phase relationships of the cycles relative to external physical ones

may be modified by appropriate treatment of the organism and will persist for a few, and often many, cycles. The endogenous cycles also may gradually drift away from their initial phase relationships when placed in constant conditions away from cyclic light and temperature changes. The rate of drift is characteristically a function of the constant illumination, or constant-temperature, level; brighter light behaves usually like higher temperature. But in some other instances the cycles show no measurable drift over long periods, behaving in their precision, therefore, more like the mean metabolic cycles. Also, cycles of quite other periods than the natural daily ones may be directly induced by light, for example, but the organism thereafter placed in constant conditions reverts at once to daily cycles.

The characteristic of essential temperature-independence of the cycle-frequencies, whether one deals with solar-day, lunar, or annual ones, made it rather improbable that the basic frequency-determining mechanism was endogenous. However, one hypothesis that has been rather widely entertained bears the assumption of the possession by the organisms of accurate, fully autonomous, endogenous biological clocks timing all the natural period lengths. Although the majority of investigators have always cautiously dissociated the possible clock mechanism from the endogenous physiological cycles studied and whose frequencies they appeared to regulate, some of the more recent supporters of this hypothesis of an autonomous endogenous clock have uncritically identified the fundamental clock system with each of the various observed endogenous and labile physiological cycles studied. On the basis of this quite unjustified assumption, the postulated clock is then usually considered to have all the properties demonstrable for these clock-regulated, and undoubtedly endogenous, cycles.

None of the several hypotheses suggested for the independent biological clock, however, have satisfactorily resolved the problem of the determination, in an essentially temperature-independent manner, the exact intervals of the turning points in the persisting cycles. So-called endogenous rhythms with single cycles ranging from 24 hours to a year in length cannot readily be conceived in terms of any fully autonomous mechanism based upon the reaction kinetics of any biochemical, or biophysico-chemical, systems with which we are now familiar.

An alternative hypothesis, advanced by Brown (1957b), proposed that the basic mechanism of temperature-independence of the frequency of biological rhythms in "constant conditions" involves the operation of a cyclic exogenous stimulus operating upon a responsive protoplasmic system, and giving rise in all cells to systematic fluctuations containing all the major natural periodisms of the external environment. These could then be readily used by the organism in timing its endogenous cycles. The latter could be considered as bearing any pre-set, fixed lag or lead relationship, or even possess a smoothly and continuously drifting relationship, thus providing endogenous cycles of frequencies differing slightly in lengths from 24 hours. The often-reported small influence of light and temperature level on cycle length in constant conditions can well operate through influencing the coupling mechanism between the exogenous basic clocks and the endogenous organismic rhythms.

We still do not know whether the exogenous daily clocks operate on a universal or local-time basis. It is even quite possible that both kinds of cyclic elements are present, each through correlation with other geophysical factors possessing one or other of these two cyclic characters, *e.g.* atmospheric electrical potential change (uni-

versal time) ; tides of atmospheric pressure (local time). The existence, however, of exogenous daily cycles has been demonstrated by means of local-time-related, aperiodic phenomena, which are in good measure superimposed on the local-time-related tides of the atmosphere.

It is evident that the solar daily cycle described in this research is clearly of solar-day rather than sidereal-day length. The possible existence of a cycle of the latter period-length was investigated. The existence of one was suggested by the annual cycle involving a positive to negative deviation about 1 AM-to-midnight linear daily trend (Fig. 2), and by the apparent gradual shifting of the solar-day maximum across the day (Fig. 3). This day, being 3.93 minutes shorter than the solar day, would be expected to scan the solar day almost exactly once each year. Similarities in the form of mean-metabolic cycles with cosmic radiation ones (Brown, Webb and Bennett, 1958) and of metabolic cycle amplitude with background radiation cycle amplitude (about half of this radiation is thought to be of cosmic-ray-origin), both give further likelihood for the existence of such a period-length in the living organism. The mean, apparent sidereal-day cycle which was found showed an amplitude of less than 2%. However, in view of the extreme closeness of the period of the year to the length of the cycle of periodic reinforcement of solar-day with sidereal-day cycles, many years of data would obviously be required to resolve the problem of the relative roles of the sidereal day and annual cyclicities in the production of the cycle depicted in Figure 1, B.

It should be emphasized that the low correlations obtained in this study in no manner imply that the correlations with the effective agent would be similarly small. Correlations between consecutive values in parallel cycles of the same frequencies would be expected to be, and are, much higher. The latter, however, could not be used to demonstrate the dependence of one cycle upon another or even on a factor correlated with the second. The cycles of the effective factor may be substantially more regular, and its correlations with organismic metabolism of a much higher order.

SUMMARY

1. Oxygen-consumption was monitored almost continuously in potatoes, *Solanum tuberosum*, in constant conditions, including pressure, for more than two years. A paralleling 8-month study of O_2-consumption in carrots, *Daucus carota*, was also made.

2. The potatoes showed an essentially bimodal mean daily cycle with an average amplitude of the major maximum of 3.7%. The cycles for the two years taken separately were very closely similar.

3. The daily cycle exhibited an annual cycle of form change, with cycles unimodal, inverted, with a 7 PM maximum in February and unimodal with an 11 AM maximum in October. The intervening months yielded bimodal cycles, with graded transitional forms.

4. The daily cycle and its annual fluctuation in the carrot resembled in great detail those obtained concurrently for the potato.

5. An annual cycle in average daily rate of O_2-consumption was found in the potato. The cycle was essentially sinusoidal with minimum in October–November and maximum, the rate about doubled, in April–May.

6. Throughout the two years the 5–6–7 AM deviation in O_2-consumption from linear daily trend was always correlated with the 2–6 AM mean rate of barometric pressure change for the same morning. The sign of this correlation exhibited a characteristic change twice each year, once in the spring and again in the fall.

7. For the two-year period of study the amplitude of the daily cycles showed a linear correlation with the concurrent outside air-temperature, with the sign of the correlation reversing about 57.5° F. With temperature expressed as deviation from 57.5° F., the coefficient of correlation was − 0.51 ± 0.049.

8. The data suggest the existence of a rhythmic component of sidereal-day length in the potatoes. Problems in its final resolution are discussed.

9. The evidence points quite conclusively to the possession by the organism, even in so-called "constant conditions," of environmentally imposed oscillations of the natural, daily and annual periods.

10. The fluctuations in the still unidentified, external effective factor appear importantly influenced by, and may possibly even in some measure determine, meteorologic changes of temperature and pressure.

11. The significance of these findings for the problem of the mechanism of the basic daily and annual biological "clock" regulating in "constant conditions" the well-known endogenous organismic rhythms is discussed at some length.

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