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CIRCADIAN ORGANIZATION AND MICROBIOLOGY: VARIANCE SPECTRA AND A PERIODOGRAM ON BEHAVIOR OF *ESCHERICHIA COLI* GROWING IN FLUID CULTURE * †

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I. INTRODUCTION. Over three decades ago Rogers and Greenbank (1) published observations on intermittent growth in a bacterial culture. These interesting data are analyzed further in this report by computational procedures (2-8) designed for detecting and evaluating the significance of nearly periodic phenomena. By such methods, circadian (about 24-hour) periodicity in a culture of *E. coli* is unmasked as a significant component of the recorded changes. These results extend the already broad scope of circadian temporal organization in microbiology.

II. BACKGROUND. On the occasion of a recent symposium, Pittendrigh (9) was asked to comment, first, on some forms of life exhibiting circadian rhythms and, second, on the nature of the observed activity in each case. He aptly replied that it is easier to list those organisms that cannot as yet be regarded as circadian systems (cf. also 10-15).

As noted elsewhere (16), the number of reliable observations on circadian periodicity over the wide range of biologic phenomena seems small, when compared to the stock of more classical physiological knowledge. However, precedents are available to suggest that circadian rhythms for many functions will eventually be detected if certain methodological difficulties are overcome (17). Actually, many sets of observations published earlier on topics thought to be unrelated to metabolic rhythms provide evidence for significant periodicity. Moreover, some sets of data, originally reported as indicating

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the absence of rhythms, have, upon revaluation, led to the opposite interpretation.

The cave crayfish *Orconectes pellucidus*, for instance, has been described as a species with an "arrhythmic activity pattern with respect to the twenty-four hour day-night cycle" (18). Recently, Brown, after revaluating the data, reported a significant activity rhythm (19). According to Ehret (20) as well as Pittendrigh (9) only viruses and bacteria can now be cited as forms of life lacking evidence for circadian organization.

Apart from bacteria and viruses, a number of unicellular and pluricellular microorganisms have already been found to show circadian periodicity (9). It is also pertinent to bacteriologists that the effect of endotoxins upon the mammalian host is a function of the latter's temporal organization: Circadian system-phase can predictably tip the scale between death or survival from the identical dose of *B. melitensis* or *E. coli* toxin (21, 22). Physiologic temporal considerations, discussed earlier at this Academy (23, 24) and elsewhere (9, 25) thus are pertinent to biologists interested in microorganisms as such or in certain microbial-host interactions.

The analyses of data on *E. coli*, to be presented herein, enlarge further the comparative physiologic domain to which temporal organization applies (9, 15, 24, 25). Along this line of thought this paper will be composed of two parts. First, the broad scope of circadian organization as an aspect of biologic time structure is sketched. A few references to earlier reviews, with emphasis upon microbiological studies, are provided. Thus the stage is set for the second part, the analysis of the important studies by Rogers and Greenbank.

III. ALIGNMENT OF SPATIAL STRUCTURE WITH TEMPORAL COUNTERPART. The widespread occurrence of periodic phenomena with a frequency of about one cycle per day illustrates a prominent aspect of biologic temporal organization. We are here dealing with the organism's dynamic structure in time which complements the more static morphology in space.

Historically, biologists have conceived of structure in terms of spatial order within a given organism, organ, tissue or cell. Modern morphologists are successfully probing more deeply into cyto-achitecture down to its molecular level. Electron-microscopists and biochemists have already joined hands in attempts to localize the arrangement of different molecules in various parts of a given cell. However, analyses of geometric relations in spatial structure as they are fostered by advances in biochemical and biophysical techniques do not usually consider the role played by temporal aspects of biologic organization. Even the circadian make-up of organisms, a rather prominent feature, has yet to be included within the scope of most contemporary studies in cellular and molecular biology.

Does this lack of concern with *temporal structure*—not to be confused with the time elapsed from start of observations (24)—represent an optimal approach? This question is equivalent to asking whether biologic structure has no dimension in time. But the signifi-

cance, reproducibility and scope of circadian rhythms (16) at least can hardly be questioned. Therefore, one must realize that biologic structure is spatiotemporal. Both these facets of organization have to be evaluated, in order to forestall undue chaos. Spatial units, such as cellular and subcellular ones, await alignment with corresponding temporal units.

IV. SCOPE OF CIRCADIAN ORGANIZATION AS A TEMPORAL UNIT OF BIOLOGIC STRUCTURE AND ACTIVITY. Circadian periodicity characterizes groups of organisms as well as the individual. Thus, for human populations there is a well-established rhythm in the incidence of birth and death, as well as in many other phenomena bracketed by these events (15, 25).

Moreover, circadian rhythms of man are not immediately or solely dependent upon the periodicity of his environment. It is hardly surprising that the patient subjected to a rigorous institutional daily routine exhibits rhythms with periods of about 24 hours; yet, so does the Strategic Air Command aviator, whose activity patterns are drastically different from 24-hour periodic ones (26).

Circadian rhythms are maintained in mammals by the temporal organization of metabolism and its controls, adrenocortical, neurosecretory, and other (15, 16). Within limits, temporal physiologic integration persists in a variety of environments. This circadian integration of body functions, while maintaining internal timing (24), also constitutes a physiologic basis for the successful adaptation of species to a prominent temporal feature of their environment on earth. The plastic circadian system is readily amenable to synchronization with certain periodic environmental factors and, of course, to phase-shifting by them (16).

In lower forms of life circadian organization is found as well, even though certain endocrines, nerves and neurosecretions are not localized in organ-systems. This has become apparent from extensive work on unicellular forms, among others, as shown in Table 1.

In the mammal, at lower levels of integration, evidence of circadian periodicity extends also to cellular and subcellular organization. By combining histologic techniques with differential centrifugation and radioactive tracer methods, rhythms are demonstrable in the mitotic activity of various tissues and in some aspects of the intracellular metabolism of a DNA, an RNA and several phospholipids. The incorporation of P^{32} into phospholipids, for instance, is found to be periodic even in liver microsomes (15).

Some controls of circadian organization, metabolic (54) and other (55, 25), have been disclosed. However, techniques corresponding to those of gland removal and replacement, used in exploring mammalian mechanisms of periodicity, are not readily applicable to problems in microbiology. Indeed, the task of analyzing the physiologic factors underlying a temporal structure in microorganisms is complicated by the crowding of diverse functions into a relatively small morphologic unit.

Such difficulties notwithstanding, work with microorganisms has

TABLE 1. Selected references to circadian organization in microorganisms

Organism:	*Function:*	*References:*
Non-Photosynthetic Protozoa		
Paramecium bursaria	Mating ability*	20, 27, 28
Paramecium bursaria	Response to ultraviolet	29
Paramecium multimicronucleatum	Mating-type expression**	30-32
Paramecium aurelia	Mating ability	33
Photosynthetic Protozoa		
Euglena gracilis	Phototaxis	34, 35
Gonyaulax polyedra	Luminescence, division, photosynthesis	36-40
Algae		
Hydrodictyon reticulatum	Photosynthesis, growth	41, 42
Oedogonium cardiacum	Sporulation	43-46
Chlamydomonas moewusii	Division	47
Acetabularia major	Photosynthesis	48
Acetabularia crenulata	Photosynthesis	48
Fungi		
Neurospora crassa	Growth (zonation)***	49-51
Monilia fructicola	Growth (zonation)	52
Armillaria mellea	Luminescence	53
Mycena polygramma	Luminescence	53
Panus stipticus	Luminescence	53
Pilobolus sphaerosporus	Sporulation	71

* Periodically, cells are capable or incapable of undergoing the process of conjunction (clumping and pair-formation). Mating type ("sex") does not change.

** Periodically, cells change mating type, being, e.g., of one "sex" by day and of the complementary type by night. Little or no change in ability to conjugate.

*** Concentric rings in growing colonies of fungi, due to alternating zones of two distinct types of growth.

some decided advantages. One can study large numbers of organisms, in a small space, at relatively little cost and for prolonged time periods and critical environmental conditions can be controlled or standardized more readily than in organisms of added complexity and size.

Moreover, study of unicellulars can provide challenging opportunities for work on periodicity. It has allowed, for instance, the elegant report by Sweeney and Haxo that the nucleus is not immediately indispensable for the maintenance of a photosynthetic rhythm in *Acetabularia* (48). Work on *Gonyaulax*, in turn, has led to basic models of enzyme-substrate interactions along the 24-hour scale, provided by Hastings (56).

Important studies of the mechanisms and consequences of circadian rhythms in higher plants have already contributed greatly to the knowledge on biologic temporal organization (11, 57, 58). An extension of such work to bacteria seems indicated.

In turning to microorganisms of lesser structural complexity than protozoa or unicellular algae, one enters a field as yet replete with semantic difficulties, reflecting, perhaps, the lack of consensus on criteria for classification. A timely exposition of this problem, references on the subject, and electronmicroscopic criteria have been provided by Ehret (59) for those concerned with important differences in subcellular organization. For the sake of brevity herein, the provision of such groupings is merely pointed out but not discussed, while the following conventional distinctions are used.

One group of microorganisms, including most algae, fungi and the protozoa, might be considered as truly cellular. These organisms possess a well-defined membrance-enclosed nucleus and a highly structured cytoplasm. In terms of classical phylogenetic order, members of this group are related to the metazoa, whose cells possess similar characteristics. The mammalian erythrocyte might be listed as a possible exception, yet this structure is a derivative of a cell with a well-defined nucleus.

Another group of microorganisms is represented by the bacterial and rickettsial forms. Organisms at this level of organization are capable of self-reproduction, in terms of both their structure and function, but their protoplast is more primitive in that the genomic apparatus and associated plasma are separated from the environment by only one envelope-system. The lowest level of biologic structural complexity may be regarded in a restricted sense as that of a virus, or more broadly as including a wider range of structures capable of self-reproduction, but devoid of a well-defined nucleus as well as a protoplast (60). Among these groupings, circadian periodic behavior has been previously described only in microorganisms possessing a well-defined nucleus.

V. SOME OBSERVATIONS ON POSSIBLE CIRCADIAN BEHAVIOR OF A BACTERIAL CULTURE. Against this background it is interesting to turn to observations on intermittent growth and/or motility in the bacterium, *E. coli*, published by Rogers and Greenbank (1).

One of the original authors, Dr. Rogers, in response to an inquiry, kindly permitted and encouraged further analysis of his data published 31 years ago. He suggested also that the experiments be repeated with modern methods for measuring and recording the growth rate of a bacterial population. In this note we endeavor to undertake the first of these tasks and thus to stimulate interest in the second.

Rogers and Greenbank were interested in bacterial growth under conditions simulating those "in the animal body, in which fresh food is constantly supplied, and by-products removed by the blood and other body fluids." They comment that growth in a long tube of fluid medium does not exactly duplicate conditions in the body but it does permit a fluid colony to extend itself continuously into regions at most only slightly affected by the previous growth. The authors then constructed such an apparatus. They used a pyrex tube, 7 mm. wide and 15 meters long, wound in a flat spiral with a flask sealed to its inner end. The tube contained a broth (1000 cc. of infusion broth, 5 gm. of peptone, and 0.5 gm. of lactose) and Bromthymol blue as indicator. This apparatus was kept in a small room maintained at 30° C throughout study.

The experiment started with the inoculation of a motile form of *E. coli* at one end of the tube. As the colony grew or moved, its progress through the tube was marked from time to time by the indicator's change in color. Turbidity also was a gauge described by the authors as "nearly identical" with "decolorization."

A motion picture camera was arranged to photograph the coiled tube at 1-hour intervals. The parameter evaluated was an approximation of colony advance—the length of the tube-section undergoing, in unit time, a change visible on the photographic record. Available for analysis is a figure with colony advance, in mm./hr., plotted on the ordinate and time on the abscissa. Hourly observations from one ex-

periment are thus graphed and extend from the 25th hour post-inoculation to the 175th hour.

In commenting on their figure, the authors state "that there is a considerable degree of periodicity to the alternation of rapid and slow growth." Their interpretation is strengthened by the added note that their figure "is representative of a number of experiments."

Rogers and Greenbank recognized that their results might be accounted for by assuming that periods of active motility alternate with periods of lesser or no activity. Since the numbers of organisms are not known, the problem whether the changes recorded indicate the colony's growth or its motility cannot be evaluated. There is no basis, in turn, for doubting the authors' interpretation that they were not evaluating acid advance.

But whatever the parameter evaluated might be, simple or complex, it appears to be nearly periodic during a 6-day period of observation. Therefore, the results deserve consideration herein, whether they indicate, as seems unlikely, acid advance or, rather, growth or motility, or, probably, an interaction of these factors. The authors' title and summary, both referring to growth, as such, should be thus qualified. As noted before, careful comments that are pertinent to the foregoing qualifications are provided in the text of the original paper.

According to a personal communication from the authors, the apparatus was kept in darkness, except that light was turned on at 1-hour intervals for photography. Whether or not lighting did play a role in bringing about the periodicity of the bacterial system cannot be decided, but it seems improbable that variations in lighting can directly and immediately account for the relatively low frequency components unmasked by the following analysis. The periodic "output" of the culture — with a non-24-hour circadian period to be demonstrated — was associated with a rather different "input" of light signals, i.e., a 1-hourly periodic "input."

VI. COMMENT ON PROCEDURES. The original data of Rogers and Greenbank (1) were unavailable. Hence, numbers read off their published graph were used. The error involved in this procedure seems negligible, in view of the magnitude of the recorded changes.

Two types of analysis were done, both by electronic computer. To forestall machine or program artifact. each of these two computer programs was checked by a test-run on other data with known results. Moreover, and apart from theoretical considerations (2-4), the use of two separate, though related, computational procedures served as a double-check on the results.

One of the procedures used was the periodogram, originally described by Schuster (3). It was computed as outlined in detail and illustrated with a simple example by Koehler et al. (6).

As to the periodogram, one should remember that for an arbitrary function it may exhibit any shape whatsoever. But if it has a well-defined maximum point, the abscissa of this point may be taken as

the estimate of a periodic component (τ) contributing to the data. The ordinate of this point is an approximation of the amplitude (C). It also is essential to note that the periodogram of the data on *E. coli* (1), as it will be here presented, represents solely a descriptive statistic.

A discussion of the sampling error of τ and C remains beyond the scope of this periodogram. But a considerable advantage derived from computing the periodogram lies in the fact that one thus hoped to obtain—and did obtain—an independent yet related validation of the estimates provided by variance spectra of the same data.

Variance spectra given with their confidence intervals represent analytical statistics. Therefore, they might be assigned more weight in the summary of analyses, to follow after some additional technical detail.

Variance spectra were computed according to Blackman and Tukey (4). This procedure is based upon generalized harmonic analysis, originally outlined by Wiener (2). The particular method employed has been indicated with a simplified computational example by Panofsky and Halberg (7, 8).

The actual computations done to obtain the variance spectra in Table 2 consisted broadly of four steps (7, 8). First, the autocovariance of the original data was computed for lags zero to m (m + 1 values). Thereafter a harmonic analysis was made of the autocovariances and the amplitudes tabulated as a function of frequency. These amplitudes, constituting the rough spectral estimates, were smoothed, in their turn, by a weighted moving average (7). Finally, the confidence intervals of each estimate were obtained.

For variance spectrum analysis original data may be transformed, first, into the autocorrelation function. This computational step seems indicated if, as in the case to be analyzed, attention is focused upon frequency. In general, the autocorrelation function has the same frequency content as the original data. Yet, whereas the original function may have any particular phase, the autocorrelation function always has a maximum at lag zero.

By making the harmonic analysis on the autocorrelation function, one computes the so-called "normalized" spectrum of a given time series, i.e., the spectrum divided by the variance. This procedure has a considerable computational advantage in that the autocorrelation function can be stopped after m autocorrelation coefficients and the harmonic analysis of this abbreviated series then provides m + 1 smoothed spectral estimates.

Variance spectra are particularly desirable, if one is dealing with so-called noisy time-series (4, 5, 7, 8, 61), that are familiar to biologists—at least by their appearance on records if not by name. Among many other graphs, a plot of the observations made by Rogers and Greenbank (1) also falls into the category of noisy time series: the periodicity of the original observations can be recognized, although it is distorted by noise (kind B-data discussed elsewhere: 61, p. 292).

In dealing with such observations, variance spectra can reduce some of the statistical uncertainties associated with the more classical direct methods of harmonic analysis. One can estimate the significance, if any, of certain components within a specifiable frequency domain.

Tukey has shown indeed that for certain noisy time series the statistical uncertainty of variance spectral estimates decreases as the ratio of the total number of observations to the m chosen for analysis increases (4). On this basis, he designed methods for determining the statistical reliability of the spectral

TABLE 2. Variance spectra, at several resolutions (m), of growth and/or motility of *E. coli* (Presumably constant conditions of temperature and nutrient, N = 150; Δ t = 1 hr)*

Period (Hours):	*Spectral Estimate:*	*Confidence Limits:*	
		0.05 Limit	.95 Limit
	m = 24		
∞	.9	.4	1.5
48.0	62.8	27.4	109.9
24.0	179.0	78.0	313.2
16.0	187.1	81.6	327.5
12.0	117.4	51.2	205.4
9.6	82.7	36.0	144.7
8.0	67.6	29.5	118.3
6.9	57.3	25.0	100.3
6.0	48.9	21.3	85.5
5.3	44.9	19.6	78.6
4.8	52.8	23.0	92.4
4.4	65.4	28.5	114.5
4.0	57.5	25.1	100.9
3.7	45.3	19.8	79.4
3.4	50.4	22.0	88.3
3.2	50.3	22.0	88.0
3.0	39.3	17.1	68.7
2.8	38.6	16.8	67.5
2.7	43.7	19.1	76.6
2.5	53.4	23.3	93.5
2.4	50.7	22.1	88.8
2.3	27.3	11.9	47.7
2.2	21.6	9.4	37.8
2.1	29.2	12.7	51.1
2.0	29.9	13.0	52.3
	m = 48		
∞	10.9	2.9	23.2
48.0	4.8	1.3	10.3
32.0	36.0	9.5	76.6
24.0	138.8	36.6	295.1
19.2	148.6	39.2	315.9
16.0	68.6	18.1	145.9
	m = 72		
∞	11.7	1.9	28.6
48.0	4.1	.7	9.9
36.0	0.0		
28.8	24.2	3.9	58.8
24.0	109.6	17.7	267.0
20.6	114.8	23.4	352.7
18.0	72.9	11.8	177.6
16.0	26.6	4.3	64.7

*Data from Rogers, L. A. and G. R. Greenbank: 1930. The intermittent growth of bacterial cultures, *Journal of Bacteriology*, 19:181.

estimates that will be used for the present purpose. Thus, in Table 2, the confidence limits can be given for each spectral estimate.

It is further pertinent to note with respect to the choice of several m's for analysis that the decision as to m determines not only the statistical uncertainty of spectral estimates, but also the resolving power of the spectrum. In other words, a small m yields estimates of greater statistical reliability than a large one, but the analysis with the larger m, while less reliable, has the greater resolving power.

VII. RESULTS OF ANALYSES. Table 2 shows variance spectra of the

data on growth and/or motility of *E. coli* (1). The complete spectrum done with m = 24 as well as the 90% confidence limits of the spectral estimates are listed on top of the table. In the domain analyzed, certain components of variation with relatively low frequency are clearly dominant over those with higher frequency. More specifically, this spectrum reveals a high "power" band between 16 and 24 hours. However, this band is quite broad, since the analysis with m = 24 provides a rather low resolution.

Accordingly, the region between 16 and 48 hours is reexamined with m = 48 and also m = 72. The spectral estimates for this particular domain only are listed in Table 2, although, of course, the entire spectrum was obtained with both m = 48 and m = 72. The complete analyses, as well as their portion shown in Table 2, reveal that the estimate around 19.2 hours, with m = 48, and that around 20.6 hours, with m = 72, are both significantly above the general level of the spectrum. The latter estimate is taken as the best available approximation of the principal periodic component in the original data.

Moreover, with m = 72, the spectrum as a whole (not shown) exhibits secondary bands with a frequency higher than that around 1 cycle/20.6 hours. But these secondary components are much less prominent than the circadian one. Thus, one must refrain from further comment beyond suggesting that work on components with a frequency much higher-than-circadian will call for more extensive data (if it is done under conditions comparable to those used by Rogers and Greenbank).

It might be reemphasized that available data did suffice, however, to reveal the band around 20.6 hours, a circumstance which attests further to the significance of the circadian component. Moreover, circadian periodicity stands out clearly not only in the variance spectrum but also in the periodogram.

The periodogram of the data on growth and/or motility of *E. coli* is shown in Figure 1. The divisions on the abscissa indicate the periods actually tested according to Schuster (3). It may be seen that over the entire interval analyzed there are periodogram estimates at 1-hour intervals. Moreover, between 18 and 26 hours such points are available at one-half hour intervals, while between 19 and 22 hours the periodogram is based upon points obtained for trial periods at intervals of 15 minutes. With this resolution, there is a well-defined maximum point between 20.5 and 21.0 hours, with the peak at about 20.75 hours.

The significant band around 20.6 hours, obtained with m = 72 by variance spectrum analysis, agrees very closely with the peak at 20.75 hours in the periodogram. The two procedures are related (2). Comparable results thus are not surprising but they do rule out computational artifact.

Since the interval between successive observations made by Rogers and Greenbank (1) is 1 hour, the original data cannot be analyzed for higher frequency changes, such as those described by Finn and Wilson (62) for population dynamics in a continuous propagator for microorganisms.

Two points can be made on the basis of the analyses. First, the original data of Rogers and Greenbank on growth and/or motility of *E. coli* contain a significant circadian component of variation. Second, the period involved, while close to 24 hours, is probably not of exactly that length.

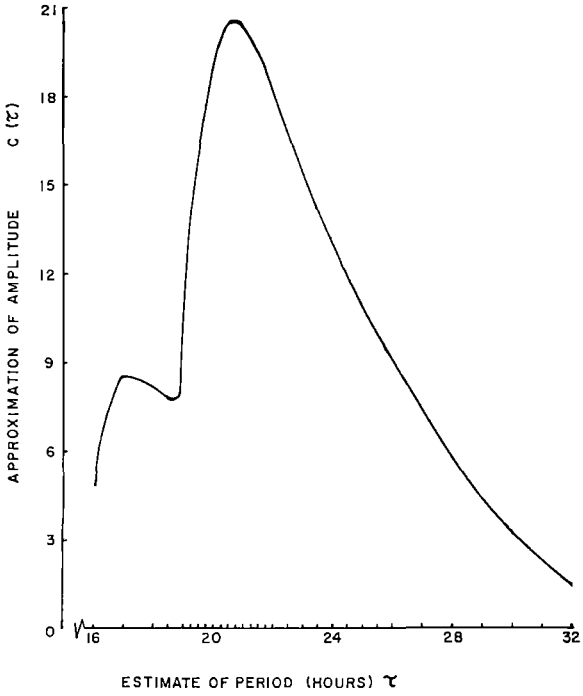

Figure 1. Periodogram of data by Rogers and Greenbank (1) on behavior of *Escherichia coli* growing in fluid culture.

VIII. COMMENT ON RESULTS. Certain environmental signals, impinging upon the organism with a 24-hour periodic schedule, readily synchronize the circadian rhythms of many species. Usually, the lighting regimen is the dominant synchronizer of these rhythms in the mouse (12, 15). The social routine plays the corresponding role for civilized man (15). In the absence of synchronizers or upon removal of their receptors, most circadian rhythms free-run (24). Periods that are close to 24 hours but not of exactly that length (9, 12, 15) are then seen.

If under presumably constant conditions, at least of nutrient and temperature, the culture of *E. coli* would have shown 24-hour synchronized circadian behavior, this result would have been difficult to interpret. It could have been solely an effect of external factors that had remained inadvertently uncontrolled (cf. 24).

If, in turn, as is the case, the period recorded for the bacterial system is circadian, yet desynchronized from the 24-hour clock (24), the rhythmic change recorded is probably not an immediate or direct consequence of environmental changes with a similar frequency.

Thus far, there is no evidence in support of a spectrum of environmental frequencies corresponding to the multitude of free-running rhythms observed in various organisms (9, 12, 16). The finding of a

desynchronized circadian rhythm, as opposed to a 24-hour synchronized one, thus supports the assumption that one is faced, at least to a significant extent, with a periodic physiologic characteristic of the organism, rather than exclusively with the organism's mere reaction to periodic external factors.

Manifold evidence from two recent symposia (63, 64) seems to be overwhelmingly in favor of the thesis that temporal biologic organization, while obviously reactive to a number of environmental influences, periodic or other, is neither the sole nor the immediate result of hidden external periodicities. The present analysis of the data published earlier by Rogers and Greenbank (1), in revealing a free-running component, apparently extends the scope of circadian organization to at least one aspect of bacterial population dynamics. Until adequate data and analyses are made available, other references to bacterial "periodicity" by students of natural selection (65-69) must remain beyond the scope of this discussion. Work and data also will be needed for a discussion of cyclic events in viruses, in addition to the interesting thought that "metastable molecules, able to undergo reversible structural alterations, might be feedback regulating mechanisms" of cyclic functions (70).

IX. CONCLUSIONS AND SUMMARY. Rogers and Greenbank demonstrated that a population of *E. coli* observed in fluid culture during a period extending from the 2nd to the 7th day post-inoculation grew and/or moved intermittently (1). With analytical statistical procedures now available for the study of the frequency content in a time series (4), this suggestion by Rogers and Greenbank can be validated. Growth and/or motility of *E. coli* in a culture made under presumably constant conditions of temperature and nutrient shows indeed "a considerable degree of periodicity" (1).

Moreover, with the procedures used, periodic component(s) can be specified and their statistical uncertainty estimated. In a variance spectrum of the original data (1), encompassing the domain from 1 cycle/2 hrs. to 1 cycle/48 hrs., as well as in a periodogram, the principal band is circadian. What seems equally important, the circadian period disclosed is not of exactly 24-hour length. Secondary bands also are seen in the variance spectrum but their computed confidence limits forestall final interpretation. More work on such components with a frequency much higher than circadian seems warranted.

It is concluded that circadian periodicity characterizes at least one instance of bacterial population dynamics. To what extent such periodic behavior results from interacting external and internal factors cannot be decided, yet the observation of a free-running circadian rhythm does suggest that factors within the bacterial population critically underlie the periodic behavior.

These findings, among many others, extend further the already broad scope of circadian rhythms in general biology. Such rhythms represent a dynamic dimension of organisms in time and can be aligned with the more conventional spatial morphology encountered

in various forms of life, including microorganisms. In this context, some of the current approaches to a temporal biology in organisms more complex than bacteria also were sketched. Emphasis was placed on simple forms of life, on unicellulars in particular. The scope of circadian organization in microbiology was thus intimated. Furthermore, some of the advantages for periodicity research associated with the use of a number of species with widely differing complexity were noted.

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