Oxygen Uptake Rhythms in a Cockroach Gauged by Variance Spectra

Physiological rhythms with several different frequencies often characterize the same set of measurements made as a function of time. With sufficient data available, such frequencies can be concomitantly and objectively evaluated by variance spectra[1-4]. By the same procedure, the statistical significance of components thus detected can also be estimated. Herein we illustrate with a pooled variance spectrum (Figure), rhythms in oxygen consumption determined by standard Warburg manometry on unselected adult male American cockroaches, *Periplaneta americana* (L.).

This work was prompted by ground-studies of DUTKY, SULLIVAN, SCHECHTER et al. aimed at a biosatellite experiment involving the telemetering of circadian (about 24 h) rhythms in motor activity and in muscle potentials from another species of cockroach, namely *Leucophaea maderae* (F.)[5]. Circadian rhythms stood out clearly in their illustrative data but the added question arose as to spectral components with higher frequency in time series from cockroaches and it seemed to deserve study prior to the use of this species as a biosensor in extraterrestrial space.

There is much earlier information on circadian rhythms in cockroaches[6-11], and as background to the present work a study by JANDA and MRCIAK can be cited[12]. These authors noted in 1957 that the activity rhythm is paralleled by changes in oxygen consumption in cockroaches, in keeping with reports on the same two rhythms in rodents[13-14].

The present work was done at three environmental temperatures (18, 24 or 30°C) on cockroaches exposed to constant light as well as to a regimen of 12 h of light alternating with 12 h of darkness. It was decided *a priori* that irrespective of the 'regularity' of the data, all series comparable in terms of temperature and lighting be pooled for the analysis of rhythms in oxygen consumption. Rhythms were explored by periodograms[15] as well as variance spectra in a broad domain ranging from circadian to much higher frequencies. The domain analyzed in the Figure extends from 40 min (0.67 h) to over 48 h.

In spectral analysis, resolution depends upon the number of lags at which the first (autocorrelation) transformation of the data is stopped, this number being designated by m[3,4]. As m is increased greater resolution is gained, though at the expense of statistical stability[1-4]. The spectra in the left half of the Figure, with $m = 36$, reveal a component with a period of about 3.4 h. This component can be said to be less prominent than the circadian one. The estimate of the latter is confounded by trends, being located as the second point from the low frequency (long period) end of the spectrum.

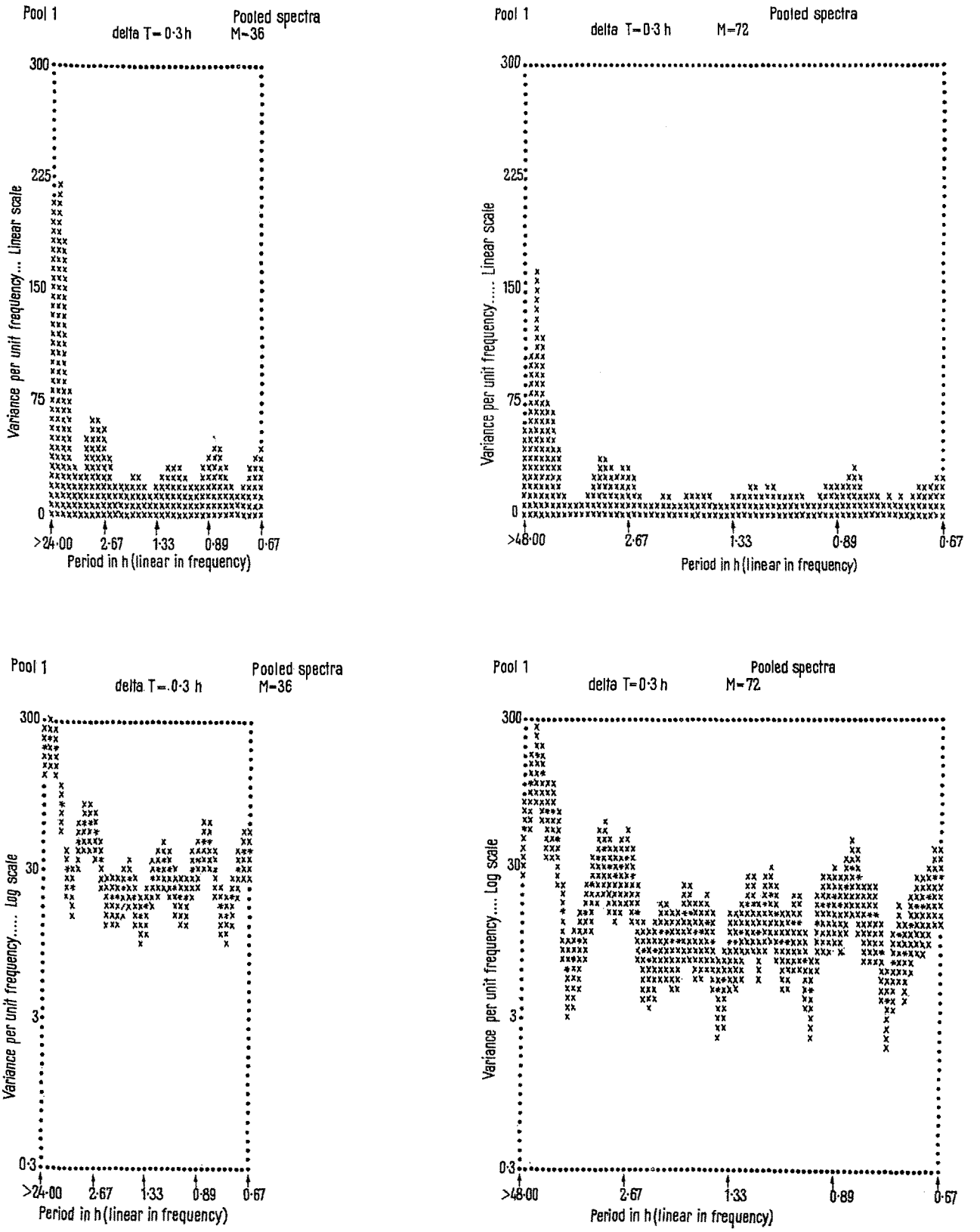
The spectra at the bottom of the Figure have a logarithmic ordinate, in lieu of the linear scale in the plots on top. In the spectra on top of the Figure, but not at the bottom, the areas under a curve (drawn to connect the tops of adjacent columns of X's) estimate the variance per unit frequency. In the plots at the bottom, spectral estimates are represented by asterisks; the lengths of the X'd columns above and below each asterisk denote the 95% and 5% confidence limits. It can be seen that a so-called ultradian component[16], of about 3.4 h, stands out significantly above the general level of the spectrum, as does perhaps also another component with a period of about 0.86 h.

The impression from spectra computed with $m = 36$, on the left of the Figure, is supported by an analysis of the same data with higher resolution ($m = 72$). The circadian component in the latter spectra, on the right in the Figure, is now the third point from the low frequency end. This resolution does not suffice to discuss the question whether the circadian component had an average period of exactly 24 h or, as would appear from the inspection of plots, a so-called free-running, non-24-h period[17]. However, spectra such as those in the Figure suffice to suggest that rhythms with a frequency higher than circadian also characterize the oxygen consumption of the cockroaches studied. Ultradian rhythms with periods of about 3.5 h actually represent wobbling frequencies and stand out as broad bands in the spectrum. Such relatively high-frequency changes have long been known from the inspection of various physiologic records (e.g. of gross motor activity in rodents) but they are difficult to quantify by the naked eye. Pooled variance spectra have revealed these broad bands in time series on several functions, ranging from telemetered interscapular rodent temperatures to steroid levels in peripheral human blood or in canine adrenal venous effluent[18].

The so-called ultradian band[16] with periods in the neighborhood of 3 or 4 h, and also a band with a period of about 0.8 or 0.9 h, were detected not only in the data summarized by the Figure on cockroaches studied at 30°C in constant light but also in spectra of cockroaches kept at temperatures of 24 or 18°C. Interpretation of the band near the high-frequency end of the spectrum is complicated, however, by aliasing, as discussed elsewhere[4].

Bands in the variance spectrum indicative of rhythms have several implications. Changes with a frequency corresponding to a given band can now be anticipated and should be controlled. Otherwise, experimental results may be difficult to interpret in any study of insect oxygen consumption whether or not one's aim includes the study of rhythms.

[1] R. B. BLACKMAN and J. W. TUKEY, *The Measurement of Power Spectra* (Dover Publications Inc., New York 1958).
[2] D. M. A. MERCER, in Cold Spring Harbor Symposia on Quantitative Biology (Long Island Biological Assoc., New York 1960), p. 73.
[3] F. HALBERG and H. PANOFSKY, Exp. Med. Surg. *19*, 284 (1961).
[4] H. PANOFSKY and F. HALBERG, Exp. Med. Surg. *19*, 323 (1961).
[5] W. N. SULLIVAN, M. S. SCHECHTER, S. R. DUTKY, and J. C. KELLER, J. econ. Ent. *55*, 985 (1962). – M. S. SCHECHTER, S. R. DUTKY, and W. N. SULLIVAN, J. econ. Ent. *56*, 76 (1963).
[6] J. S. SZYMANSKI, Pflügers Archiv. ges. Physiol. *158*, 343 (1914).
[7] D. L. GUNN, J. exp. Biol. *17*, 267 (1940).
[8] K. MELLANBY, J. exp. Biol. *17*, 278 (1940).
[9] J. L. CLOUDSLEY-THOMPSON, Annu. Mag. nat. Hist. *6*, 705 (1953).
[10] J. E. HARKER, J. exp. Biol. *33*, 224 (1956).
[11] E. BÜNNING, *Photoperiodism and Related Phenomena in Plants and Animals* (American Association for the Advancement of Science, Washington, D.C. 1959), p. 507.
[12] V. JANDA and M. MRCIAK, Mém. Soc. zool. tchécosl. *21*, 244 (1957).
[13] A. HEUSNER, J. Physiol. (Paris) *49*, 205 (1957).
[14] A. HEUSNER, C.R. Soc. Biol. *153*, 1258 (1959).
[15] F. KOEHLER, F. K. OKANO, L. R. ELVEBACK, F. HALBERG, and J. J. BITTNER, Exp. Med. Surg. *14*, 5 (1956).
[16] *Circadian Systems*, Report of the Thirty-ninth Ross Conference on Pediatric Research (Fomon, S. J., ed., Ross Laboratories, Columbus, Ohio, USA, 1961).
[17] F. HALBERG, in, Cold Spring Harbor Symposia on Quantitative Biology (Long Island Biological Assoc., New York 1960), p. 289.
[18] J. GALICICH, E. HAUS, F. HALBERG, and L. A. FRENCH, Ann. N. Y. Acad. Sci., in press (1963).



Pooled variance spectra, summarizing in several ways a total of 657 measurements of oxygen consumption (made at 20 min intervals on four mature male cockroaches, *Periplaneta americana* (L.), kept at 30°C in continuous light (about 10 lux) without food or water). Abscissae are linear in frequency but are labeled as reciprocals of frequency, i.e. as period. Ordinates represent variance per unit frequency, plotted linearly on top and logarithmically at bottom. Resolution is lower in plots on the left ($m = 36$), higher in plots on the right ($m = 72$). Vertical bands in the spectrum suggest rhythms with corresponding periods (see text). (For computational details see HALBERG and PANOFSKY, Exp. Med. Surg. *19*, 284 (1961)).

Furthermore, students of physiological rhythms need not restrict their attention to the circadian component by viewing only its presence or absence in the raw data. In a variance spectrum components which are quite irregular on inspection of the raw data can also be objectively quantified and bands in the higher-than-circadian domain of frequencies are actually of interest to a more complete analysis of circadian system physiology. Factors underlying shifts of variance from the circadian component into the domain of adjacent frequencies seem to be of particular interest[19–21].

Zusammenfassung. Der Sauerstoffverbrauch von *Periplaneta americana* (L.) wurde im Dauerlicht und im 24-stündigen Licht-Dunkelwechsel bei 30, 24 oder 18°C alle 20 min bestimmt. Im Varianzspektrum dieser Beobachtungsreihen lassen sich neben der ungefähren Tagesperiodik (Circadian-Periodik) auch höherfrequente, unregelmässigere und weniger prominente Rhythmen nachweisen.

A. G. RICHARDS and F. HALBERG

Departments of Pathology and Entomology, University of Minnesota (Minneapolis, U.S.A.), September 6, 1963.

[19] F. HALBERG, Proc. Royal Soc. Med. *56*, 253 (1963).

[20] Mr. M. DIFFLEY carried out all analyses by electronic computer; Misses M. HOLMES and B. RESAR and Messrs. F. JONES, W. KRINGEN, H. LOFGREN and D. PAULSON helped competently with manumetric readings at 20-minute intervals for over three weeks. Prompt procurement of equipment by Mr. F. DUCKERT, Assistant Purchasing Agent, Physicians & Hospitals Supply Co., Minneapolis, and Mr. C. SMITH, Purchasing Agent, University of Minnesota, made the work feasible.

[21] Work supported by the National Aeronautics and Space Administration (NsG-517) and the United States Public Health Service (GM-06671, C-4359 and NB-04531-02).

Vasopressin Analogues with Selective Pressor Activity

It is generally recognized that the basicity of the amino acid residue in position 8 of the molecule, i.e. in the penultimate position of the peptide side-chain, is of considerable importance for the specific biological properties of the vasopressins. This has been pointed out for both the pressor[1] and the antidiuretic[2] effect. In continuation of our studies on the influence of small structural modifications on the pharmacological properties of the neurohypophysial hormones[3,4], some new analogues modified in the position 8 have been synthesised and investigated biologically. Of these compounds, ornithine[8]-vasopressin, phenylalanine[2]-ornithine[8]-vasopressin, ornithine[8]-oxytocin and phenylalanine[2]-ornithine[8]-oxytocin proved to be particularly interesting. The present paper gives a short account of their synthesis[5] and main pharmacological properties.

Nα-CBO-Nδ-tosyl-L-ornithine was condensed with ethyl glycinate by the dicyclo-hexylcarbodiimide method to yield ethyl Nα-CBO-Nδ-tosyl-L-ornithyl-glycinate. After removal of the CBO protecting group, this dipeptide was condensed with N-CBO-L-proline by the same method. The product, ethyl N-CBO-L-prolyl-Nδ-tosyl-L-ornithyl-glycinate, was converted by amidification to the corresponding amide. Removal of the CBO protecting group and condensation with N-CBO-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-azide[6] afforded N-CBO-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide. After removal of the CBO protecting group this hexapeptide was condensed with *p*-nitrophenyl N-CBO-S-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalaninate[7] to N-CBO-S-benzyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide, with *p*-nitrophenyl N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-phenylalanate[7] to N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide, with *p*-nitrophenyl N-tosyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucinate[8] to N-tosyl-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide and with *p*-nitrophenyl N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-isoleucinate[7] to N-CBO-S-benzyl-L-cysteinyl-L-phenylalanyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nδ-tosyl-L-ornithyl-glycinamide.

Removal of the protecting groups of the four above-mentioned nonapeptides by treatment with sodium in liquid ammonia, followed by oxidation with potassium ferricyanide, purification by counter-current distribution in the system sec-butanol/water/trifluoroacetic acid (120:160:1), conversion to the acetate and lyophilization, yielded Orn[8]-vasopressin, Phe[2]-Orn[8]-vasopressin, Orn[8]-oxytocin and Phe[2]-Orn[8]-oxytocin respectively. These four peptides were proved to be pure by different chromatographic and electrophoretic methods and they gave correct elementary analysis and amino acid composition on hydrolysis.

The main pharmacological effects of these peptides were determined by comparing them with the Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances[9]. Both the pressor and the antidiuretic potencies were assayed in rats: the former on the blood pressure of animals in urethane anaesthesia after pretreatment with an adrenergic blocking agent[10,11], the

[1] P. G. KATSOYANNIS and V. DU VIGNEAUD, Arch. Biochem. Biophys. *78*, 555 (1958).

[2] W. H. SAWYER, W. Y. CHAN, and H. B. VAN DYKE, Endocrinology *71*, 536 (1962).

[3] R. A. BOISSONNAS, ST. GUTTMANN, B. BERDE, and H. KONZETT, Exper. *17*, 377 (1961).

[4] B. BERDE, *Pharmacologie des hormones neurohypophysaires et de leurs analogues synthétiques* (Masson, Paris 1963).

[5] For a detailed account on the synthesis of Orn[8]-vasopressin and of Orn[8]-oxytocin see: R. HUGUENIN and R. A. BOISSONNAS, Helv. chim. Acta *46*, 1669 (1963).

[6] R. A. BOISSONNAS, ST. GUTTMANN, P.-A. JAQUENOUD, and J.-P. WALLER, Helv. chim. Acta *38*, 1491 (1955).

[7] R. L. HUGUENIN and R. A. BOISSONNAS, Helv. chim. Acta *45*, 1629 (1962).

[8] P.-A. JAQUENOUD and R. A. BOISSONNAS, Helv. chim. Acta *45*, 1462 (1962).

[9] D. R. BANGHAM and M. V. MUSSETT, Bull. Org. mond. Santé *19*, 325 (1958).

[10] J. DEKANSKI, Brit. J. Pharmacol. *7*, 567 (1952).

[11] British Pharmacopoeia (Pharmaceutical Press, London 1958).