PRO NATURA INTEGRA

Agreement in Endpoints from Circadian Rhythmometry on Healthy Human Beings Living on Different Continents

In an attempt to determine whether a given value is 'normal' or 'abnormal', clinicians as well as biologists customarily study many rhythmic variables on the basis of single samples – without qualifying such values in terms of the phases of the various rhythms contributing to the variable sampled, even though it has been pointed out that a single datum can meaningfully be qualified as to circadian system phase[1]. Herein we document that from relatively few samples – some of them on variables extensively utilized by biologists and clinicians – reasonably reliable new and potentially useful endpoints of rhythms can be obtained for several physiologic variables[1–4].

This note does not dwell on the kinds of subjects providing the samples analyzed, nor on their age, sex, ethnic background or nutritional state. The biophysical or biochemical methods used in sampling are largely ignored, as are the circumstances of observation to be standardized, as indicated earlier[2]; but while such details remain beyond our scope, they obviously are indispensable for routine work and will have to be thoroughly considered in any specific rhythmometric study of amplitudes as well as levels. In this note the amplitude serves primarily for the weighting of the acrophase, defined as the crest of the function(s) used for approximating a rhythm.

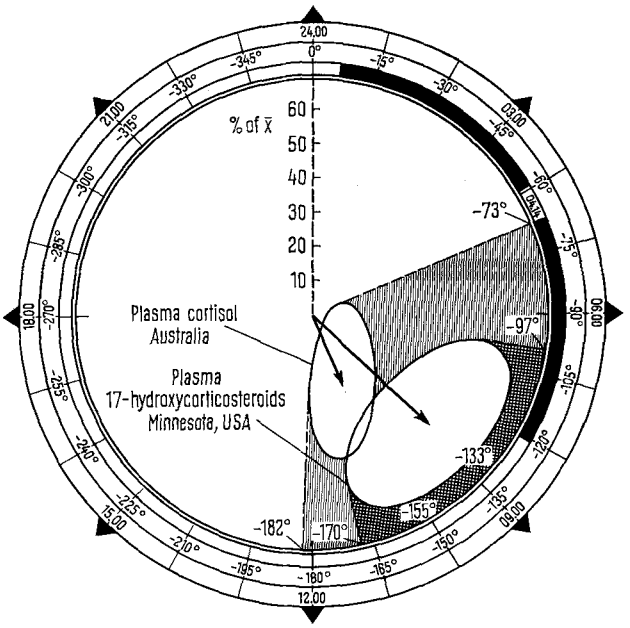
Plasma corticosteroids. Data from 2 groups of subjects, both living on a routine of diurnal activity and nocturnal rest, reveal similar external timing[5] for the rhythm in plasma 17-hydroxycorticosteroid (17-OHCS). 1 group of 20 healthy adult white male subjects was studied in 1958 at Minnesota by sampling at intervals of about 3 h over a day and a half; blood was withdrawn through indwelling venous catheters and the plasma 17-OHCS were determined according to the method of Wu and Mason[6].

In a second study[7], plasma cortisol was determined by the double isotope derivative procedure of Kliman and Peterson[8], with a similar sampling schedule involving 3-hourly intervals over 30 h. This work was done in 1963 on 8 healthy adult male whites living in Australia.

Estimates of the acrophase (crest) and of the amplitude of the best-fitting 24-h cosine function – with local midnight serving as phase reference – were obtained for each series in each study by means of a computer program providing a least squares fit of harmonic functions[9]. These imputations served for the computation of a vector sum and an error ellipse by the cosinor program[10], separately on the samples of short time series from Minnesota and on those from Australia, as shown in the Figure.

The Figure reveals the extent of agreement in the timing of the circadian acrophases in plasma 17-OHCS and cortisol in these studies carried out with different methods on 2 continents several years apart.

It can be seen, first, that neither of these error ellipses overlaps the center of the plot, the so-called pole. This finding indicates that both populations exhibit a statistically significant circadian rhythm of plasma cortisol. The acrophase of the rhythm occurs during the local forenoon in the data from Australia as well as in those from Minnesota. The acrophase estimates in the Figure are determined objectively from all available data, rather than by subjectively inspecting the temporal location of crests in 'zig-zags' representing time plots of original data – the chronograms. Of course, the so-called 'microscopic' evaluation of the circadian component in the Figure does not contradict the 'macroscopic' impression from the chronogram; rather it serves for objective quantification.



Agreement in results of circadian rhythmometry on certain adrenocortical hormones in human blood analyzed by cosinor. In each case, 0° is midnight local clock time. Neither error ellipse overlaps the pole; both samples thus are characterized by statistically significant circadian rhythms. The overlap of 95% confidence arcs indicates failure to detect a statistically significant difference between the circadian acrophases in the 2 studies[3]. Synchronizer schedule for the month preceding sampling in Australia shown on outer circular scale. Corresponding values for the Minnesota study (not shown) are: 'lights off', 23.00 (−345°) and 'lights on', 07.00 (−105°).

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Table I. Circadian rhythm of human hydroxycorticosteroid and potassium excretion in samples of time series from different geographic locations

Site of study	No. of subjects (No. of days) [Δt, h]	Noise to signal [SE″/C]	*P* rhythm detection	Circadian Amplitude, C (95% confidence limits)	Circadian Acrophase Φ[a] (95% confidence limits)	Author(s) Reference
Hydroxycorticosteroid (mg/h)						
USA (Minnesota)	4 (5) [2–9]		< 0.03	0.15 (0.04–0.26)	−129° (− 83 to − 200)	HALBERG and HAUS[b]
(Minnesota)	8 (1) [3]	0.21_8		0.12 (0.06–0.17)	−153° (− 128 to − 177)	DOE[21]
(Maryland)	7 (2) [3]		< 0.002	0.11 (0.06–0.16)	−154° (− 123 to − 181)	BARTTER et al.[23]
(Pennsylvania)	9 (1) [8]		< 0.002	0.09 (0.04–0.14)	−144° (− 104 to − 183)	CURTIS et al.[24]
	9 (1) [8]		< 0.009	0.16 (0.05–0.26)	−143° (− 128 to − 168)	
(Minnesota)	1 (34) [3.3]		< 0.005	0.17 (0.13–0.20)	−130° (− 117 to − 144)	HAUS and HALBERG[22]
Mexico	1 (20) [4, 8]	0.12_5		0.05 (0.04–0.06)	−159° (− 145 to − 173)	PENA[b]
	1 (12) [4, 8]	0.26_8		0.03 (0.01–0.05)	−133° (− 103 to − 163)	
	1 (8) [4, 8]	0.16_0		0.10 (0.08–0.12)	−112° (− 77 to − 130)	
	1 (9) [4, 8]	0.19_6		0.09 (0.07–0.11)	−110° (− 87 to − 132)	
	1 (12) [4, 8]	0.18_0		0.07 (0.05–0.09)	−118° (− 109 to − 139)	
	1 (6) [4, 8]	0.24_3		0.04 (0.02–0.06)	−115° (− 105 to − 142)	
South Dutch Guiana	10 (2) [2]		< 0.005	0.08 (0.05–0.11)	−169° (− 143 to − 192)	HALBERG and SIMPSON[20]
	10 (2) [2]		< 0.005	0.11 (0.07–0.15)	−164° (− 134 to − 202)	
Scotland	10 (2) [2]		< 0.005	0.18 (0.06–0.23)	−165° (− 152 to − 175)	HALBERG and SIMPSON[20]
	10 (2) [2]		< 0.005	0.19 (0.11–0.28)	−157° (− 133 to − 182)	
France	12 (1) [4]		< 0.005	0.07 (0.05–0.09)	−138° (− 121 to − 155)	REINBERG[b]
	6 (2) [4]		< 0.01	0.08 (0.04–0.12)	−146° (− 115 to − 176)	REINBERG et al.[25]
	6 (2) [4]		< 0.025	0.06 (0.01–0.11)	−135° (− 77 to − 191)	
	7 (1) [4]		< 0.009	0.11 (0.04–0.19)	−157° (− 125 to − 192)	GHATA et al.[b]
Germany	9 (2) [3–15]		< 0.001	0.15 (0.08–0.22)	−143° (− 127 to − 195)	HALHUBER et al.[b]
	9 (2) [3–15]		< 0.007	0.18 (0.07–0.29)	−142° (− 121 to − 178)	
Austria	10 (3) [1.5, 10]		< 0.05	0.9 (0.1–1.6)	−166° (− 89 to − 223)	GÜNTHER et al.[26]
	10 (25) [1.5, 10]		< 0.005	3.6 (2.4–4.7)	−173° (− 150 to − 194)	
	10 (25) [1.5, 10]		< 0.005	3.7 (2.1–5.3)	−188° (− 165 to − 206)	
	10 (25) [1.5, 10]		< 0.005	4.5 (2.8–6.2)	−176° (− 154 to − 195)	
	10 (3) [1.5, 10]		< 0.005	2.8 (1.3–4.4)	−169° (− 139 to − 196)	
Italy	31 (1) [2]		< 0.001	0.17 (0.15–0.19)	−134° (− 124 to − 143)	CERESA[27]
Ceylon	7 (1) [4]		< 0.007	0.14 (0.08–0.20)	−133° (− 111 to − 184)	GHATA et al.[b]
Thailand	13 (1) [6]	0.39_2		0.06 (0.02–0.10)	−150° (− 106 to − 194)	MAROTTA et al.[28]
Australia (tetrahydro-F)	8 (1) [~3]		< 0.002	0.06 (0.03–0.09)	−103° (− 85 to − 133)	GORDON et al.[7]
Potassium (mEq/h)						
USA (Minnesota)	1 (52) [3.3]	0.13_5		1.6 (1.1–2.2)	−171° (− 156 to − 186)	HAUS and HALBERG[22]
(Minnesota)	5 (5) [4, 8]		< 0.005	1.3 (0.7–1.9)	−161° (− 98 to − 202)	HALBERG[b]
(Minnesota)	8 (1) [3]		< 0.001	2.1 (1.4–2.8)	−157° (− 130 to − 181)	DOE[21]
(New York)	8 (1) [4, 9]		< 0.001	1.4 (0.9–1.9)	−117° (− 95 to − 171)	KRIEGER[31]
Mexico	5 (14) [3–8]		< 0.05	0.5 (0.04–0.9)	−165° (− 94 to − 242)	GARCIA SAINZ[b]
	1 (20) [4, 8]	0.06_6		1.6 (1.4–1.8)	−152° (− 145 to − 160)	PEÑA[b]
	1 (3) [4, 8]	0.33_4		1.7 (0.6–2.7)	−172° (− 135 to − 210)	
	1 (12) [4, 8]	0.17_1		1.4 (1.0–1.9)	−145° (− 126 to − 165)	
	1 (8) [4, 8]	0.17_1		1.4 (0.9–1.9)	−124° (− 104 to − 143)	
	1 (9) [4, 8]	0.18_8		2.1 (1.4–2.9)	−114° (− 93 to − 135)	
	1 (12) [4, 8]	0.72_6		0.5	−182°	
	1 (6) [4, 8]	0.16_7		2.0 (1.4–2.7)	−126° (− 107 to − 145)	
	1 (3) [4, 8]	0.28_8		0.8 (0.4–1.2)	−129° (− 96 to − 161)	
France	1 (21) [2–7]	0.11_1		1.2 (0.9–1.4)	−159° (− 146 to − 172)	REINBERG[b]
	7 (1) [4]		< 0.002	1.6 (0.8–2.3)	−140° (− 104 to − 158)	GHATA et al.[b]
	4 (1) [4]		< 0.025	1.3 (0.6–2.1)	−151° (− 87 to − 189)	REINBERG et al.[30]
Germany	9 (2) [3–15]		< 0.002	1.0 (0.5–1.5)	−149° (− 125 to − 215)	HALHUBER et al.[b]
	9 (2) [3–15]		< 0.02	1.6 (0.4–2.8)	−156° (− 126 to − 189)	

Austria	10 (3) [1.5, 10]	< 0.01	1.6 (0.8–2.3)	− 158° (− 132 to − 177)	GÜNTHER et al.[26]
	10 (25) [1.5, 10]	< 0.01	1.8 (1.2–2.4)	− 155° (− 139 to − 174)	
	10 (25) [1.5, 10]	< 0.01	1.5 (1.0–2.1)	− 150° (− 137 to − 174)	
	10 (25) [1.5, 10]	< 0.01	1.5 (0.8–2.2)	− 140° (− 123 to − 167)	
	10 (3) [1.5, 10]	< 0.01	1.3 (0.6–1.9)	− 150° (− 117 to − 187)	
Ceylon	7 (1) [4]	< 0.002	1.4 (0.8–2.0)	− 174° (− 154 to − 231)	GHATA et al.[b]
Australia	8 (1) [~3]	< 0.001	2.6 (1.6–3.3)	− 123° (− 92 to − 143)	GORDON et al.[7]
	5 (1) [4]	< 0.05	1.6 (0.1–2.0)	− 133° (− 102 to − 138)	GHATA et al.[b]

[a] τ = 24 h = 360°; 1 h = 15°. Φ reference = middle of habitual sleep span. [b] Unpublished.

Table II. Circadian rhythm of several systemic functions in samples of human time series from different geographic locations

Site of study	No. of subjects (No. of days) [Δt, h]	Noise to signal [SE″/C]	*P* rhythm detection	Circadian			Author(s) Reference
				Amplitude, C (95% confidence limits)	Acrophase	Φ[a]	
Oral temperature (°C)							
USA (Minnesota)	1 (725) [~8]	0.05_0		0.14 (0.13–0.15)	− 200° (− 193 to − 206)		HALBERG[b]
(Minnesota)	1 (20) [2–12]	0.26_6		0.11 (0.05–0.17)	− 229° (− 199 to − 258)		KOGL[b]
(Minnesota)	1 (39) [3–24]	0.16_7		0.18 (0.12–0.24)	− 197° (− 178 to − 216)		HALBERG[b]
(Maryland)	14 (12–30) [6]		< 0.001	0.21 (0.13–0.28)	− 200° (− 179 to − 221)		BARTTER et al.[23]
(Minnesota)	5 (5) [4, 8]		< 0.01	0.28 (0.24–0.32)	− 203° (− 191 to − 209)		HALBERG[b]
	1 (5) [4, 8]	0.12_2		0.34 (0.26–0.42)	− 205° (− 191 to − 219)		
	1 (5) [4, 8]	0.17_9		0.29 (0.19–0.39)	− 197° (− 177 to − 217)		
	1 (5) [4, 8]	0.18_0		0.27 (0.17–0.37)	− 204° (− 184 to − 224)		
	1 (5) [4, 8]	0.14_3		0.31 (0.22–0.40)	− 205° (− 189 to − 221)		
	1 (5) [4, 8]	0.35_8		0.22 (0.07–0.37)	− 195° (− 155 to − 235)		
(Minnesota)	11 (1) [1.5]		< 0.005	0.27 (0.20–0.33)	− 199° (− 181 to − 220)		HALBERG
Mexico	5 (15) [3–8]		< 0.03	0.16 (0.04–0.27)	− 221° (− 199 to − 345)		GARCIA SAINZ[b]
	1 (16) [4, 8]	0.10_7		0.28 (0.22–0.34)	− 246° (− 234 to − 258)		PENA[b]
France	1 (21) [2–10]	0.07_1		0.50 (0.42–0.58)	− 185° (− 174 to − 196)		REINBERG[b]
	7 (1) [4]		< 0.006	0.18 (0.07–0.29)	− 183° (− 135 to − 219)		GHATA et al.[b]
Austria	10 (3) [1.5, 10]		< 0.005	0.23 (0.13–0.33)	− 225° (− 207 to − 268)		GÜNTHER et al.[26]
	10 (25) [1.5, 10]		< 0.005	0.26 (0.19–0.33)	− 219° (− 204 to − 236)		
	10 (25) [1.5, 10]		< 0.005	0.22 (0.13–0.31)	− 231° (− 210 to − 256)		
	10 (25) [1.5, 10]		< 0.005	0.27 (0.18–0.35)	− 222° (− 205 to − 238)		
	10 (3) [1.5, 10]		< 0.025	0.31 (0.08–0.53)	− 194° (− 171 to − 221)		
Ceylon	7 (1) [4]		< 0.02	0.13 (0.03–0.22)	− 157° (− 125 to − 202)		GHATA et al.[b]
Australia	8 (14) [~3, 8]		< 0.005	0.27 (0.23–0.31)	− 215° (− 198 to − 232)		GORDON et al.[7]
	5 (1) [4]		< 0.004	0.14 (0.08–0.20)	− 205° (− 150 to − 255)		GHATA et al.[b]
Heart rate (beats/min)							
USA (Minnesota)	5 (5) [4, 8]		< 0.01	8.5 (3.1–13.8)	− 210° (− 187 to − 273)		HALBERG[b]
France	1 (21) [2–10]	0.11_3		9.3 (7.2–11.4)	− 219° (− 203 to − 236)		REINBERG[b]
Austria	10 (25) [1.5, 10]		< 0.01	2.7 (1.7–3.7)	− 206° (− 160 to − 236)		GÜNTHER et al.[26]
	10 (25) [1.5, 10]		< 0.01	2.6 (1.1–4.1)	− 228° (− 198 to − 263)		
	10 (25) [1.5, 10]		< 0.01	2.5 (0.7–4.3)	− 225° (− 195 to − 291)		
	10 (3) [1.5, 10]		< 0.05	3.9 (1.1–6.6)	− 196° (− 169 to − 277)		
Time estimation (arbitrary units)							
USA (Minnesota)	1 (516) [~8]	0.19_3		2.7 (1.7–3.7)	− 2° (− 341 to − 24)		HALBERG[b]
	5 (5) [4, 8]		< 0.001	5.2 (4.7–5.8)	− 7° (− 344 to − 98)		HALBERG[b]
	10 (25) [1.5, 10]		< 0.01	3.6 (0.8–6.4)	− 18° (− 355 to − 64)		GÜNTHER et al.[26]
	10 (25) [1.5, 10]		< 0.01	4.5 (1.7–7.2)	− 34° (− 19 to − 42)		
	10 (25) [1.5, 10]		< 0.01	5.3 (2.7–7.9)	− 20° (− 14 to − 72)		

[a] τ = 24 h = 360°; 1 h = 15°. Φ reference = middle of habitual sleep span. [b] Unpublished.

The reproducibility of the estimates of external timing may be judged by tangents drawn to the error ellipses, indicating the variability encountered in each study. This point is visualized by the two shaded areas in the Figure. Whatever the method of analysis used, it can again be noted that the acrophase of the 24-h-synchronized circadian rhythm in plasma cortisol occurs relatively early during the span of habitual activity. In diurnally active men on both continents, as in the nocturnally active mouse[11] or rat[12–15], a circadian periodic adrenal cortical activation starts during the habitual rest span; thus, it is 'preparatory' for the next activity phase[11,16] (rather than an immediate 'response' to external stimuli), just as certain uterine changes during the sex cycle are 'preparatory' to ovulation and the egg's fertilization and implantation[16].

Rhythmometry on other human data, carried out with the cosinor method by LUNEDEI, CAGNONI et al.[17], reveals considerable agreement with the results of the 2 studies summarized in the Figure, even though conditions of observation, sampling schedules and chemical methods are not the same.

Urinary corticosteroid and potassium excretion, body core temperature, heart rate and time estimation. The results of rhythmometry on urinary corticosteroid and potassium determinations from several independent studies are shown in Table I; the corresponding summary of data on body core temperature, heart rate and the ability to estimate the passage of 2 min correctly is presented in Table II. While the comparison of levels and to some extent that of amplitudes of, say, urinary corticosteroid excretion must await the standardization of biochemical techniques used, the agreement among acrophases is satisfactory for all of these variables.

Body temperature, the clinician's old mainstay, gains new meaning from rhythmometry, in that it is not only a periodic variable in its own right[33], but can also serve rather generally as a reference standard for phase to which the acrophases of other functions can eventually be related. From this viewpoint, it is particularly important to note from Table II the stability of the circadian body temperature acrophase in healthy subjects. In one case only, namely, in the cosinor summary on 5 subjects, each studied for 5 days, shown in row 5 of Table II, the same data are presented twice, since the same subjects also are dealt with individually in rows 6–10 of this Table. The stability of the oral temperature acrophase when data are available for 5 days only thus comes to the fore. For the 11 subjects, each studied for only 1.5 days, summarized by cosinor in row 11 of Table II, the variability of individual acrophases is only slightly larger (not shown here).

Obstacles to rhythmometry. The agreements reflected in the Figure and in the Tables here presented must not prompt complacency. We need diagnoses for the individual, yet we are dealing partly with group studies summarized by cosinor – a circumstance apparent from the column entitled 'No. of subjects' in Tables I and II. But in several cases we *are describing endpoints for single subjects.* The endpoints themselves are 'imputed'[10] in a spectral window by least squares; the confidence arc for the acrophases is obtained on the basis of the noise-to-signal ratio by a method described earlier (see pages 40–41 in reference [10]).

Results shown stem from the fit of a single cosine curve; these values are intended only as first rough approximations to be complemented by endpoints from other procedures, notably when the waveform of a rhythm deviates from a sinusoidal shape. The application of appropriate methods will require more frequent sampling than is currently practiced. The technology for this purpose seems to be readily available.

But even when such desiderata are met, several kinds of difficulties might prompt clinicians to refrain from collecting and utilizing information on rhythms.

(a) In many instances, there will remain the difficulty of deriving reliable endpoints from the macroscopic inspection of time plots, the so-called chronograms.

(b) An earlier obstacle may be encountered in other cases, namely the inability to carry out serial determinations, such as those of corticosteroids, sufficiently promptly to permit decisions at the bedside.

(c) Even when the foregoing obstacles are overcome, the excessive cost of serial determinations as compared to that of a single analysis may lead one to limit the number of observations.

Interpretation of results. Even students of rhythms, much less clinicians trained in the classical homeostatic approach, do not as a rule attempt to isolate a rhythm from appropriately collected data; they rely instead on a 'macroscopic' inspection of their raw data or upon averages plotted as a function of time, with at most the classical dispersion indices. So long as they do not involve biologic 'noise' removal, such studies can be labeled in a terminology used earlier[18,19] as 'macroscopic' rather than 'microscopic'.

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Such macroscopic displays in the time domain may suffice for the detection, but not for the measurement, of rhythms in data from experimental animals kept under rigorously standardized conditions. By standardizing the circumstances of observation, one tends to reduce variability – also referred to as 'biologic noise' – whether it depends upon genetic factors, the past history of the individual or outside influences impinging randomly upon the organism. The rhythms of certain laboratory rodents then stand out clearly. However, for certain clinical or field studies such standardization can be carried out only up to a certain point. For instance, control of genetics or remote past history may be impractical or undesirable. Biologic noise removal, such as that undertaken for arriving at the results of the Figure and Table I by the use of mathematical filters, then becomes as essential as is, of course, a standardization of the conditions of observation and sampling preferably by international agreements.

Delay in results reaching the clinician. Some of the problems of so-called 'turn-around time', i.e. the time needed for completing the chemical work-up of the data (apart from the time needed for the completion of numerical analysis), can be solved by autoanalyzer methods that are quickly and efficiently applicable to relatively large numbers of urine samples. However, until a more direct sensing of physiologic variables may become possible for biophysical functions and for substances in blood and urine, there remains the task of collecting reliable samples repeatedly over appropriately long spans. In this connection it should be possible to develop chemosensors comparable in performance to that of biophysical sensors already available for monitoring variables such as body core temperature. If this technologic task can eventually be accomplished, many of the difficulties and hurdles now faced by both subject and observer engaged in rhythmometry as well as the cost associated with the collection of biochemical data for rhythmometry will be greatly reduced. A few steps toward such goals have already been taken under the sponsorship of the National Aeronautics and Space Administration of the United States by the development of an automated miniaturized 'wet-chemistry' system for the analysis during space flight of a primate's urine[29].

Cost. Disregard of rhythms appears reasonable upon economic grounds; it is expensive to analyze more than a single sample. Furthermore, on a single sample of tissue, blood or urine, modern biochemists can perform many diverse determinations; it would be exceedingly costly to do all of these on serial samples. The temptation is great to exploit the opportunity for a multi-variable approach on single samples (whether or not the variables investigated are pertinent to the condition under study) rather than to focus upon rhythms in one or more pertinent variables. 'Multa sed non multum' may result from the attitude that, in the absence of pertinent information allowing the singling out of one or a few functions, one should study all possible variables in single samples – even though some, or perhaps all, of the variables already are known to be rhythmic.

Whenever many determinations are done on more than 1 sample from a given patient, the necessary laboratory work-up and the numerical analyses are indeed costly. Up to now, laboratory technology has developed in such a way that it is often as easy to carry out 12 different analyses on, say, a single sample of blood as it is to determine a single variable. In certain laboratories, the autoanalyzers are routinely programmed to perform 12 kinds of determinations, whether or not they are desirable or requested. (It should be possible to schedule the use of available instrumentation to do routinely a given kind of, say, biochemical determination on 12 different samples – for rhythmometry – at no greater cost than that of 12 different biochemical analyses on a single sample.)

In the study of the source of a disease or in the following of its course, including the effects of treatment, sampling must often be repeated at intervals; this renders the cost of the many and diverse laboratory procedures particularly excessive – even without any consideration of rhythms. It then seems again justifiable in practice to ignore rhythms so long as definite indications for rhythmometry have not been established – if for no other reason than for the sake of practicability and economy, since this approach taxes not only the capacity of a laboratory but also the resources of the patient.

Inferences. Rhythmometry could be facilitated and acquire more general usefulness if an international forum could establish agreement on phase reference, the appropriate number and scheduling of samples, the kinds of biochemical, biophysical and behavioral methods used for measurements, and the circumstances of observation, including the choice of diets. An early discussion of such problems and others[32] is planned within the framework of a chronobiologic project now being considered for incorporation into the International Biologic Program.

Despite its obvious limitations stemming from heterogeneous methods and samples, the evidence illustrated in the Figure and Tables I and II suffices to indicate that a rigorous assessment of the extent to which rhythmometry can clarify problems of general biology on the one hand, and can contribute to medical practice on the other hand, is overdue. Against the background of information derived from single samples, endpoints of rhythms can now actually be tested for their usefulness, e.g. in diagnosis, with respect to indications for treatment, and for an optimal timing of medication.

Should rhythmometry prove to be useful for any one of these aims, it will then become the superior procedure and the more economical approach. Even costly studies repeated over months or years may not detect from single samples a rhythm alteration that might be clarified by applying rhythmometry only once.

The technology of rhythmometry is within the 'state of the art' – both the methods for data collection and, more importantly, the techniques for numerical analysis.

The necessary change in concepts underlying attitudes and actions in the biologist's laboratory as well as at a patient's bedside is perhaps the major remaining obstacle. More specifically, the evidence here presented should not be interpreted as a mere suggestion to control, say, the time of day of sampling! In the long run, the assumption that endpoints of rhythms in variables such as plasma cortisol concentration can be ignored – an assumption that continues to be made in planning 'homeostatic' laboratory work, with or without control of the clock hour of sampling – may be much more expensive and more wasteful than rhythmometry, not only of material resources but also of human health and life.

In healthy human beings, the acrophases (crest-phases) of certain circadian rhythms in several systemic functions and in substances of blood and urine can objectively and readily be determined by electronic computation as will be eventually also the level, the amplitude and the waveform of a rhythm. These acrophases agree remarkably well in studies carried out by different investigators working many years and miles apart with differing biophysical, biochemical and behavioral methodology, under dissimilar standardization of the conditions chosen for observation and of the kind and extent of sampling.

This agreement among endpoints suggests that it is time for planning at an international level of the conditions and techniques that may be adopted generally to obtain standardized endpoints of rhythms in health and to check out these procedures in different laboratories around the world, with a view toward subsequent, broader-scale study of alterations of rhythm in various diseases[34].

Zusammenfassung. Circadiane Akrophasen – Gipfel der ungefähren 24-h-Periodik – von Blut- und Harnkortikosteroiden, Kalium im Harn, Körpertemperatur, Pulsrate und 2-min-Schätzung wurden mittels elektronischer Anpassung einer Kosinusfunktion vermöge der Methode der kleinsten Quadrate bestimmt. Solche Charakteristika circadianer Rhythmen empfehlen sich als Referenzstandarde dem Mediziner und Biologen durch ihre zufriedenstellende Übereinstimmung in Daten von Untersuchungen auf verschiedenen Kontinenten mit zum Teil unterschiedlichen Methoden.

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