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Temporal correlation of some endocrine circadian rhythms in elderly subjects *

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The aim of this chronobiological study was to investigate temporal correlations in the circadian patterns of 6 hormones, namely somatotrophic hormone (STH), prolactin (PRL), cortisol (F), aldosterone (ALD), insulin (IRI) and C-peptide (CP), assayed in systemic blood serum drawn at 07:00, 10:00, 13:00, 16:00, 19:00 and 22:00 h from an antecubital vein in 19 young subjects (aged 20–29 yr, comprising 10 males and 9 females; and 20 elderly subjects (aged 70–81 yr, comprising 10 males and 10 females).

All subjects were sampled on a normal dietary sodium intake (120–140 mEq/24h) while following a social routine of diurnal activity (07:00–23:00) and nocturnal rest (23:00–07:00).

Time-qualified data were analyzed by lead-lag correlation and by cosinor analysis.

According to the lead-lag correlation findings, it would appear that the correlation which exists between several time-qualified series in young subjects is no longer present in elderly subjects. The circadian rhythms which were found to have lost their temporal correlations with advancing age were those between STH and IRI, STH and ALD, PRL and IRI, PRL and CP, and ALD and CP.

It should be noted that the correlation between hormonal rhythms breaks down mainly on account of a peculiar age-related change in the magnitude of the circadian fluctuation.

This chronological decline in amplitude led to the conclusion that the senescence of endocrine rhythmic functions is a biological phenomenon characterized by altered circadian variability.

(Key words: Aldosterone, Ageing, Biorhythm, Cortisol, C-peptide, Insulin, Prolactin, Somatotrophic hormone)

Introduction

Although it may be legitimate to view endocrine function as a separate subsystem, any complete assessment must take into account a complex network of hormonal interactions. In human adults, positive correlations have been reported in the case of

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insulin, somatotrophic hormone, cortisol, prolactin and aldosterone in studies based on the 24-h time scale [1–6].

However, little is known about the endocrine interrelationships in the elderly, although changes in hormone concentrations with advancing age have been described. Increased concentrations of plasma insulin [7] and decreased concentrations of plasma aldosterone [8–10] have been reported. Prolactin concentrations were found to be unchanged by Yamaji et al. [11] and Touitou et al. [12], whereas Vekemans and Robyn reported them as being consistently lower [13]. Other investigators have noted an absence of nocturnal prolactin peaks in a number of aged subjects [14] and an increase in the 24-h mean prolactin concentration in aged men as compared with that in adult controls [15].

Although statistically significant effects of age on plasma cortisol were reported to be absent in a study based on sampling at specified clock times [16], other chronobiological studies have documented an age-dependent decrease in the circadian amplitude of cortisol patterns in aged subjects [17–19]. Age-related changes in the spontaneous secretion of somatotrophic hormone on the 24-h time scale were observed by Finkelstein et al. [20].

In this paper we compare correlations between hormones in young and elderly subjects. An account of circadian variability is given as a follow-up to our earlier work on aging and aldosterone based on Pearson product-moment correlations for 6 hormones.

Definition of terms used

Acrophase (ϕ). Timing of the highest point in a rhythm cycle defined by a mathematical model (e.g. cosine) applied to all available data (rather than to a possible single, extreme, chance value) and referred to a specific local time (midnight for the purpose of this study).

Amplitude (A). One-half of the difference between the highest and lowest values in a rhythm cycle defined by a mathematical model (e.g. cosine) applied to all available data (rather than a possible single, extreme, chance value).

Circadian. Occurring in approximately 24-h periods — from the Latin 'circa' (about, approximately) and 'dies' (day or 24 h). This adjective is used to describe both (macroscopic) variations on the 24-h time scale and (microscopic) rhythms established after rejection of the zero-amplitude assumption that are characterized by a frequency of one cycle in a 24 ± 4-h period or similar frequency. The term also describes rhythms or variations with approximately 24-h cycle length (24 ± 4 h), whether these are frequency-synchronized with acceptable environmental schedules (24-h or other time scale) or are desynchronised from or free-running in regard to local time.

Macroscopic approach. Based on scrutiny of original data or of averages and dispersion indices plotted as a function of time, without performing analyses to provide inferential statistical point and interval estimates of rhythm characteristics.

Mesor (M). Acronym denoting mean value of a rhythm defined by a mathematical model (e.g. cosine) — midline estimating statistic of rhythm.

Microscopic approach. Objective quantitative resolution of temporal characteristics of biological data, e.g. by testing the fit of a mathematical model with a time series to obtain inferential statistical point and interval estimates of rhythm and trend characteristics.

Population mean-cosinor method. A method used to estimate population rhythm parameters on the basis of the respective parameter estimates obtained from subjects by the single-cosinor method.

Rhythm. Periodic component of (biological) time series demonstrated by inferential statistical mean, preferably with objectively quantified characteristics, i.e. frequency (f), acrophase (ϕ), amplitude (A), mesor (M) and/or waveform (W). Rhythms thus include any set of biological changes recurring systematically according to an algorithmically formulatable pattern or waveform which can be validated in inferential statistical terms. Mathematically, more or less sinusoidal rhythms can be described by the use of approximating functions such as those having the form: $y(t) = M + A \cos(\omega t + \phi)$, where ω is the angular frequency and t = time.

Single-cosinor method. A method used to estimate rhythm parameters (M, A, ϕ) by the least-squares fit of the cosine curve with a single time-series of data.

Subjects and methods

A group of elderly, clinically healthy subjects from Würzburg, F.R.G., comprising 10 men and 10 women aged 70–81 yr, volunteered and gave their informed consent to participate in this investigation. The control group of young subjects was made up of medical students from Würzburg University, and comprised 9 females and 10 males aged 20–29 yr.

As shown in Table I, subjects of comparable height and weight were selected in order to avoid potential discrepancies in pancreatic hormone secretion related to overweight.

The subjects were investigated on a normal sodium intake of about 120 mEq/24 h while following a social routine of diurnal activity (from approximately 07:00) and nocturnal rest in darkness (from approximately 23:00) with meals at approximately 08:30 (continental breakfast), 12:00 (light lunch), and 18:30 (dinner). Since the subjects had no recorded impaired glucose tolerance, the diet was normocaloric, with a normal balance of carbohydrates, fats and protein in relation to weight. Possible hormonal dissimilarities linked to food intake were thus excluded.

Venous blood samples were taken as 3-h intervals (07:00, 10:00, 13:00, 16:00, 19:00 and 22:00) in order to cover 15 h of a 24-h span. This routine was dictated by the availability of elderly subjects to give blood samples at home in their normal environment. Within the 3-h interval schedule, the time that elapsed between meals and blood sampling varied from 30–90 min. However, the relationship between meal times and sampling times was always the same in each individual group investigated. The first sample, at 07:00, was taken soon after the subjects got up.

Serum was stored frozen until radioimmunoassay (RIA) was performed for aldosterone (ALD), cortisol (F), somatotrophic hormone (STH), prolactin (PRL),

TABLE I

SUBJECTS INVESTIGATED

Group	Sex	*n*	Age (yr)	Height (cm)	Weight [a] (kg)	Plasma		
						Na^{+} [b] (mEq/l)	K^{+} [c] (mEq/l)	Creatinine [d] (mg/dl)
Young	Male	10	27.2 ± 0.66	173.3 ± 2.33	70.1 ± 2.56	143.1 ± 0.80	4.0 ± 0.07	1.1 ± 0.04
	Female	9	23.1 ± 0.63	167.8 ± 2.90	63.4 ± 4.77	140.2 ± 0.92	3.9 ± 0.09	0.9 ± 0.05
Elderly	Male	10	75.2 ± 1.09	171.1 ± 3.39	75.2 ± 6.78	139.4 ± 0.71	4.3 ± 0.14	1.2 ± 0.08
	Female	10	73.7 ± 0.94	156.8 ± 1.81	61.2 ± 3.50	142.5 ± 0.56	4.3 ± 0.24	1.2 ± 0.20

[a] Rounded to nearest lower or higher integer for hectograms < 5 or > 5 respectively.

Normal ranges:

[b] 135–145 mEq/l;

[c] 3.5–5.0 mEq/l;

[d] 0.7–1.4 mg/dl. Data given as mean ± SE.

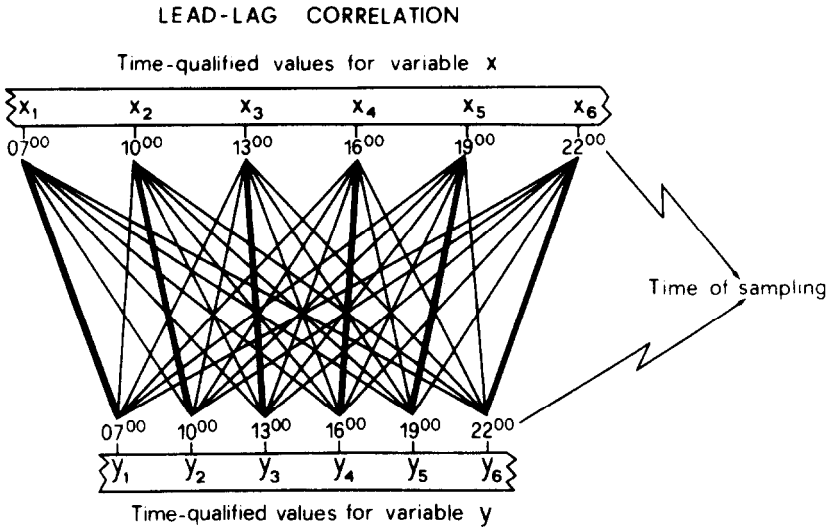

Fig. 1. Diagram showing how lead-lag correlation was applied to the statistical analysis of interrelationships among the hormones investigated in clinically-healthy young and elderly subjects. ▬, Isophasic correlation; ———, heterophasic correlation with lag displacement of 1–5 lags (1 lag = 3-h difference in blood-sampling time).

insulin (IRI) and C-peptide (CP). Various kits were used for the RIA measurements, viz., CEA-IRE-SORIN for ALD and IRI, Novo Industri for CP, Diagnostic Products Corporation for F, and Serono Diagnostic for STH and PRL. Pearson's

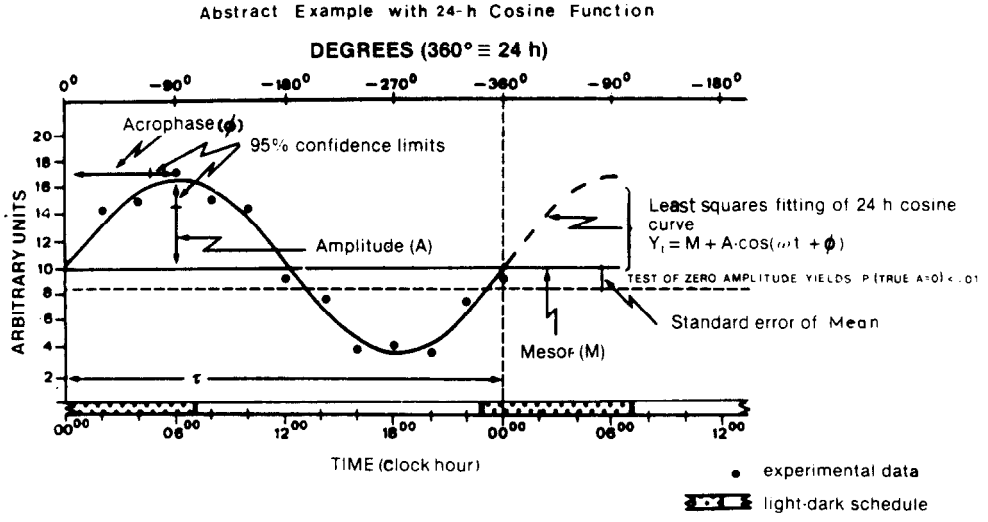

Fig. 2. An illustrative example of the cosinor chronobiological method for quantifying rhythm parameters by the least-squares fit of a 24-h cosine function with time-qualified data.

product-moment correlations (lead-lag correlation) were computed from the original data, with and without displacement of the series for one variable in relation to the series for the next variable (Fig. 1).

In addition, time series from each subject were tested for fit with a 24-h cosine function according to the single-cosinor procedure [21] (Fig. 2).

The results were summarized using the population mean-cosinor method [22] to estimate the characteristics (mesor, amplitude and acrophase) of the circadian hormonal rhythms. Differences in the cosinor-derived rhythmometric findings in old and young subjects were tested by multivariate analysis using Hotelling's t^2-procedure to compare vectorial units.

The cosinor procedure was used systematically, since the best-fitting cosine curve makes it possible to derive inferentially the nocturnal segment that is lacking in the experimental data and to define the cyclic pattern of the periodic variables while avoiding the 'biological noise' produced by momentary and/or episodic changes that so frequently affects the temporal patterns of somatotrophic hormone, prolactin and cortisol.

Results

The time-qualified mean values of the serum hormones assayed are shown in Fig. 3. The chronograms indicate that the time-qualified values of the hormonal series assayed in the elderly subjects are not comparable with the corresponding hormonal curves for the young controls. The differences in the temporal profiles are not only quantitative but also qualitative, involving the shape of variability in relation to time. Because of this pecularity, the temporal interrelationships among the hormonal series seem to be substantially different in young and old individuals. The lead-lag correlation revealed a statistically significant positive correlation be-

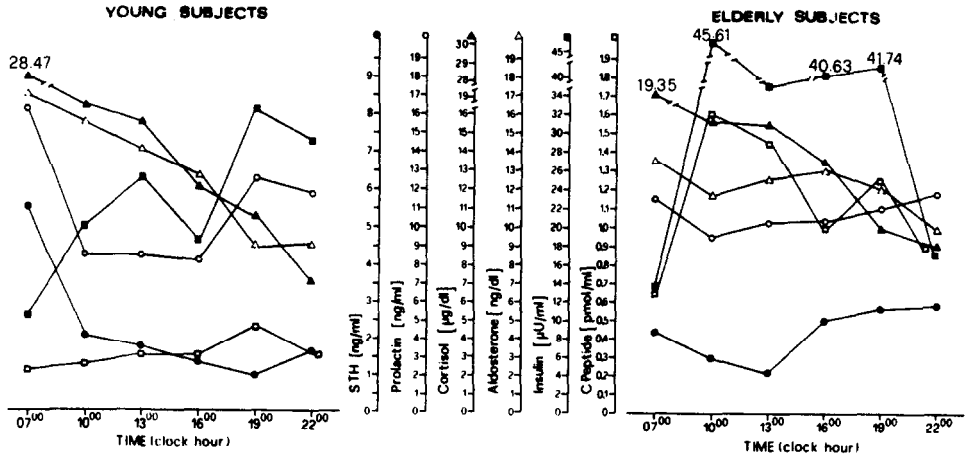

Fig. 3. Chronograms showing time-qualified mean values of the hormones investigated in clinically healthy young and elderly subjects.

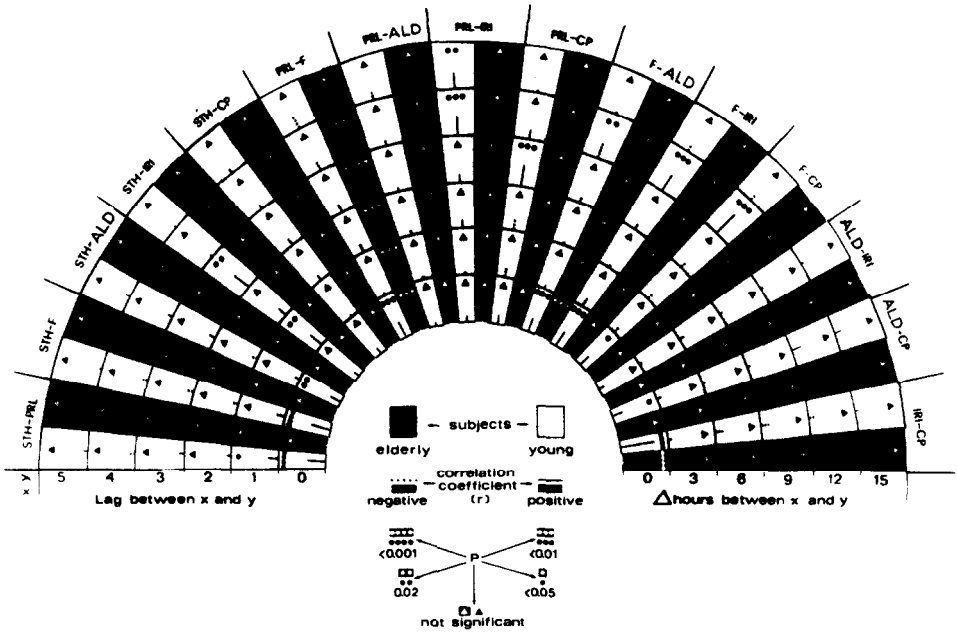

Fig. 4. Comparison of the results obtained by lead-lag correlation between time-qualified values for pairs of variables. The white segments relate to the young subjects and the black to the elderly. The length of the dotted and continuous radiating lines indicates the correlation coefficient value. Statistical significance is indicated by the number of heavy dots. The triangles denote correlations that are not significant.

tween ALD and CP, ALD and F, ALD and STH, STH and F, STH and PRL, PRL and F, F and IRI, F and CP and CP and IRI in the young subjects, and between ALD and F, PRL and F, and IRI and CP in the elderly subjects (see Fig. 4).

Accordingly, there is no evidence to indicate that correlation exists between ALD and STH, ALD and PRL, ALD and IRI, STH and F, STH and PRL, F and IRI, F and CP, IRI and STH, IRI and PRL, PC and STH, PC and ALD, or PC and PRL in elderly individuals. However, F and STH and F and CP show a correlation between values displaced by about 9 h, while STH and PRL, and F and IRI show a correlation between values shifted by about 3 h.

The cosinor-derived circadian parameters are shown in Fig. 5. Histograms indicate that the characteristics of the circadian rhythms in the elderly are not comparable with those of the circadian cycles in young people. It is interesting to note that circadian rhythms may be seen to vary in one parameter, such as amplitude, without any change occurring in the others, i.e. mesor and/or acrophase. According to the probability (P) values obtained from the statistical comparison (Hotelling's t^2 test) of the cycles in the young and elderly subjects (Table II), the rhythmometric changes found to be significant in the elderly were the decrease in circadian amplitude for ALD, F and PRL; the increase in amplitude and mesor for CP; and the shift in phase for IRI, CP, F, STH and PRL. The increase in circadian mesor and amplitude

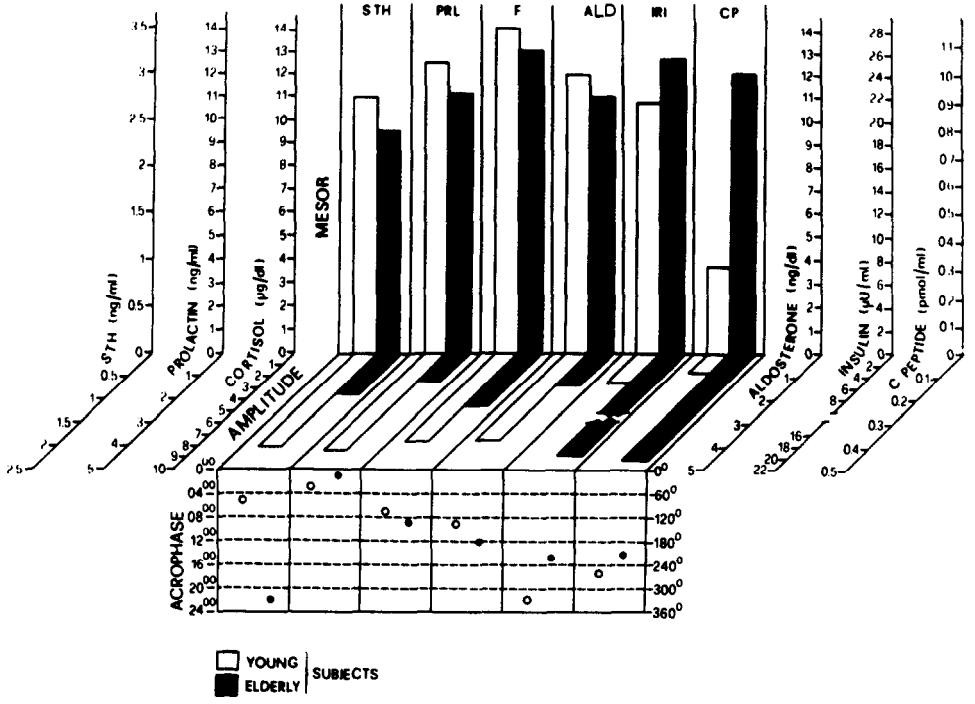

Fig. 5. Diagrammatic representation of the circadian properties of the hormonal rhythms investigated in clinically healthy young and elderly subjects.

for IRI was found to be of borderline statistical significance.

The P values determined in the statistical comparison (t-test) of the cycles in which temporal correlation was absent in elderly subjects (Table III) revealed

TABLE II

PROBABILITY (P) VALUES DETERMINED BY HOTELLING'S t^2 TEST IN THE STATISTICAL COMPARISON OF THE RHYTHMOMETRIC PROPERTIES OF CIRCADIAN RHYTHMS IN YOUNG AND ELDERLY SUBJECTS.

Hormonal rhythm	Parameters compared [a]		
	M	A	M+A+ϕ
ALD	NS [b]	0.05	NS
IRI	NS	NS	0.03
CP	< 0.01	< 0.01	< 0.01
F	NS	< 0.01	0.03
PRL	NS	< 0.01	< 0.01
STH	NS	NS	0.02

[a] M, Mesor; A, Amplitude; ϕ, Acrophase;
[b] NS, not significant

TABLE III

PROBABILITY (P) VALUES DETERMINED BY t-TEST IN THE STATISTICAL COMPARISON OF AMPLITUDES AND ACROPHASES IN THE TEMPORALLY UNCORRELATED CIRCADIAN RHYTHMS IN ELDERLY SUBJECTS AS REVEALED BY LEAD-LAG CORRELATION.

Temporally uncorrelated rhythms	Parameters compared	
	Amplitude (% of M) [a]	Acrophase (degrees)
ALD/STH	< 0.01	NS [b]
PRL/CP	< 0.001	NS
PRL/IRI	< 0.001	NS
STH/IRI	NS	NS
ALD/CP	NS	< 0.01

[a] M, Mesor,

[b] NS, not significant.

significant differences principally in the case of the amplitude and only to a much more limited extent in that of the acrophase.

Discussion

The aim of the investigation was to explore the temporal interrelationships among a number of endocrine rhythms in the elderly. Its scope was restricted to only 6 hormones assayable in human serum, although a more extensive study might have provided fuller knowledge of endocrine correlations in the later decades of life.

Correlation analysis of the hormonal series chosen was performed not only on morning fasting levels but also on a further 5 values recorded in the late morning, afternoon and evening. Nocturnal data were intentionally excluded, since the investigation covers certain circadian variables that are notoriously influenced by sleep, such as cortisol [23–27], prolactin [28–31] and somatotrophic hormone levels [32–35]. It is in fact difficult to find a group of elderly subjects whose sleep patterns are comparable. Moreover, the quantity and the quality of nocturnal sleep in a group of young control subjects and in an elderly study group naturally differ considerably.

Our study revealed that some temporal interrelationships that are detectable in the hormonal cycles of young subjects are no longer present in the elderly. This phenomenon is illustrated by the temporal correlation patterns between aldosterone and somatotrophic hormone, aldosterone and prolactin, somatotrophic hormone and C peptide, insulin and somatotrophic hormone, prolactin and insulin, prolactin and C peptide, aldosterone and insulin, and aldosterone and C peptide. Temporal correlation in elderly subjects is maintained only in the cases of aldosterone and cortisol, cortisol and prolactin, insulin and C peptide, cortisol and insulin, cortisol and C peptide, cortisol and somatotrophic hormone and somatotrophic hormone and prolactin.

The statistical analysis that was performed showed that the hormonal cycles

whose temporal correlation breaks down in the elderly are characterized by significant differences in circadian properties. In this regard, the rhythmometric parameter that was found to be most significantly discordant was the circadian amplitude, i.e. the 24-h variation from the 24-h mean level. On the other hand, the endocrine rhythms that showed an age-related concordance in their amplitude change (a decrease in the case of the aldosterone, cortisol and prolactin cycles and an increase in that of insulin and C-peptide) were found to remain correlated in elderly subjects.

Basically, the study demonstrated that the age-related change in the extent (rather than the periodicity) of circadian fluctuation plays an essential role in determining the pattern of certain endocrine interrelationships in the later decades of human life.

The results of other chronobiological studies provide convincing evidence that a decrease in circadian variability characterizes the senile patterns of several rhythmic variables, such as steroid, catecholamine, peptide, protein and electrolyte levels [12,36–43].

In view of this broader documentary evidence, it would seem likely that the chronological decline in the amplitude (amplitude clinospectrometry) of biorhythmic functions constitutes the principal phenomenon which characterizes physiologic ageing. If this is so the major problem that remains is to find a biological explanation for the age-related changes in the extent of circadian fluctuating functions which are not characterized by a decline. The key question is whether or not the age-associated increase in amplitude represents a cause or an effect of ageing.

A response to this question can be attempted on the basis of the findings reported in this paper. The reduction in aldosterone, cortisol and prolactin circadian fluctuation is conceivably a sign of functional decay. The reduction in amplitude can thus be regarded as a primary manifestation of ageing. By contrast, the amplification of insulin and C-peptide circadian fluctuation is not a sign of functional involution. The increase in amplitude can therefore be viewed as an event that is associated with ageing. It can accordingly be argued that the senile changes in circadian oscillating functions are not of equal biological significance as regards human ageing. The biological functions that show a chronological decrease in their circadian oscillating activity can be regarded as being causative of senescence, while those showing an increase with advancing age can be viewed as being adaptative to senescence. Accordingly, the biological picture of physiologic ageing may be defined as the phenotypic expression of deterministic as well as epiphenomenal changes in circadian oscillating functions.

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