Circadian Rhythm in Duration of Salicylate Excretion Referred To Phase of Excretory Rhythms and Routine.* (31863)

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Biologic responses to various agents, including drugs, vary as a function of circadian system phase(1-4) in human beings as well as in experimental animals(5). Such rhythms can readily be quantified in so-called cosinor analyses(6-8), prepared by electronic computer. The circadian amplitude, C, (or rather the double C) computed by this method is a measure of the extent of change; the timing of the rhythm is indicated, in turn, by the phase, φ.

The present report extends findings on time-varying biologic phenomena to the duration of the urinary excretion of salicylate following oral administration of this drug. The rhythmic excretion of the drug is displayed in a cosinor. The same display also evaluates more quantitatively than was heretofore possible the reproducibility in separate sampling profiles of the C and φ of 2 previously well-known prominent rhythms, namely, those in the excretion of 17-hydroxycorticosteroid (17-OHCS) and potassium(K).

Materials and methods. Three healthy female human volunteers, 22, 31 and 43 years of age, and 3 males, 18, 39 and 44 years of age, weighing 62, 60 and 63 and 54, 72 and 80 kg, respectively, were studied. All subjects lived on a routine of diurnal activity and nocturnal (23:00 to 07:00 local time, Paris, France) rest for at least one week prior to a given test. Each subject voided his bladder before taking the drug. A crystallized sodium salt of salicylate in the fixed dose of one gram was administered in 4 separate tests done on each subject at intervals of at least one week; in each of these 4 tests, the drug was administered at a different time point, *i.e.*, at 07:00, 11:00, 19:00 or 23:00.

In each test, urine was collected at 4-hour intervals for 48 hours following drug administration. The sequence in which different test times were chosen for salicylate administration was varied from one subject to the next, except that tests at 07:00 and 19:00 were completed on all subjects before tests at 11:00 and 23:00 were added.

To obtain a physiologic reference to circadian system-phase at the time of salicylate administration (apart from clock hour), urine was sampled at 4-hour intervals for 48 hours from each subject prior to each test involving drug administration at 07:00. In these urine samples, 17-hydroxycorticosteroid(9) and potassium(10) were determined. Irrespective of the sequence in which such 48-hour profiles prior to the test involving drug administration at 07:00 were obtained, the corresponding results will be labeled herein as (circadian-system-phase-) reference series I. Urines also were obtained on each subject at 4-hour intervals for 48 hours prior to salicylate ingestion at 19:00—the corresponding results being labeled herein as (circadian-system-phase-)reference series II.

Such 48-hour pre-drug-administration profiles were not obtained prior to tests involving salicylate administration at 11:00 or 23:00. The two profiles were deemed sufficient to validate the reproducibility of the circadian system phase from one test to the next, as will become apparent from results, analyzed by cosinor.

All urines were stored frozen at —30°C. Salicylate in each sample was determined by 2 closely related methods(11,12), after extraction with dichloroethane. Results of these procedures on each 4-hour sample were averaged, as if they were chemical duplicates.

The time course of salicylate excretion as it may be influenced by the circadian system phase at the time of drug administration was expressed in two ways. On the one hand,

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a) the duration of drug excretion was evaluated as the time span elapsed from time of administration to the end of the last 4-hour urine collection span associated with a sample containing more than 5 mg of salicylate per sample of urine. On the other hand, b) the amount of salicylate excreted during the first 4 hours after administration at each test time was separately analyzed.

For statistical analyses the a) or b) values obtained in different tests carried out one or several weeks apart were regarded as describing test results obtained during an idealized "day," whereby "secular" variation was admittedly ignored. Furthermore, the data for the groups of 3 male and 3 female subjects were regarded as replications, since a sex difference, if any, was not readily apparent in the small samples on hand.

The series of 4 a) or b) values were first summarized by a time plot, as were also the data on excretion of 17-OHCS and K. The same series were analyzed thereafter by the cosinor method, carried out by electronic computer(6,7). A first step involved the fit by least squares of a 24-hour cosine function of the form: $y_t = C_o + C \cdot \cos(\omega t + \varphi)$, where y_t is the a) [or b)] value at time t for a given subject, C_o is the level, C the amplitude, and φ the phase; ω, the angular frequency fitted for the purpose of these analyses, is $2\pi/24$. In other words, for this kind of analysis, the angular frequency, ω, is kept constant as 1 cycle in 24 hours.

This first cosinor step, carried out separately for each kind of series from each of the 6 subjects, yielded for a given series and subject an "imputed"(7) phase, φ', and an imputed amplitude, C'. From these pairs of values (C', φ') an amplitude, C, and the C-weighted group phase, φ, as well as the corresponding confidence regions computed with several confidence coefficients were obtained in a second step of electronic computation. These confidence regions were then drawn as error ellipses in a third automatic computing step.

By this cosinor method, a rhythm has been detected if, and only if, the error ellipse does not overlap the pole of a cosinor display(6). Furthermore, when the ellipse does not overlap the pole, the same cosinor also serves for parameter estimation, namely, for obtaining the confidence interval of the C and the confindence arc of the φ.

Results. The time plot at the top of Fig. 1 shows that the duration of salicylate excretion—index a)—lasted for 22 $\pm$ 1.36 hours (mean and standard error) when the drug was administered at 07:00; for drug administration at 19:00 the corresponding result was 17.3 ($\pm$ 1.33) hours. Tests at other times yielded intermediate a) values, as is also apparent from the top of Fig. 1.

As for results on salicylate excretion during the first 4-hour span following drug administration—index b)—these values, on the average, were higher in tests involving salicylate administration at $\approx$ 19:00 than in tests with salicylate administration at 07:00. Thus, at a time when the drug was eliminated more promptly, *i.e.*, at the circadian system phase associated with high values for excretion during the 4-hour span immediately following salicylate administration, the overall duration of salicylate excretion, as gauged in this study, was shorter.

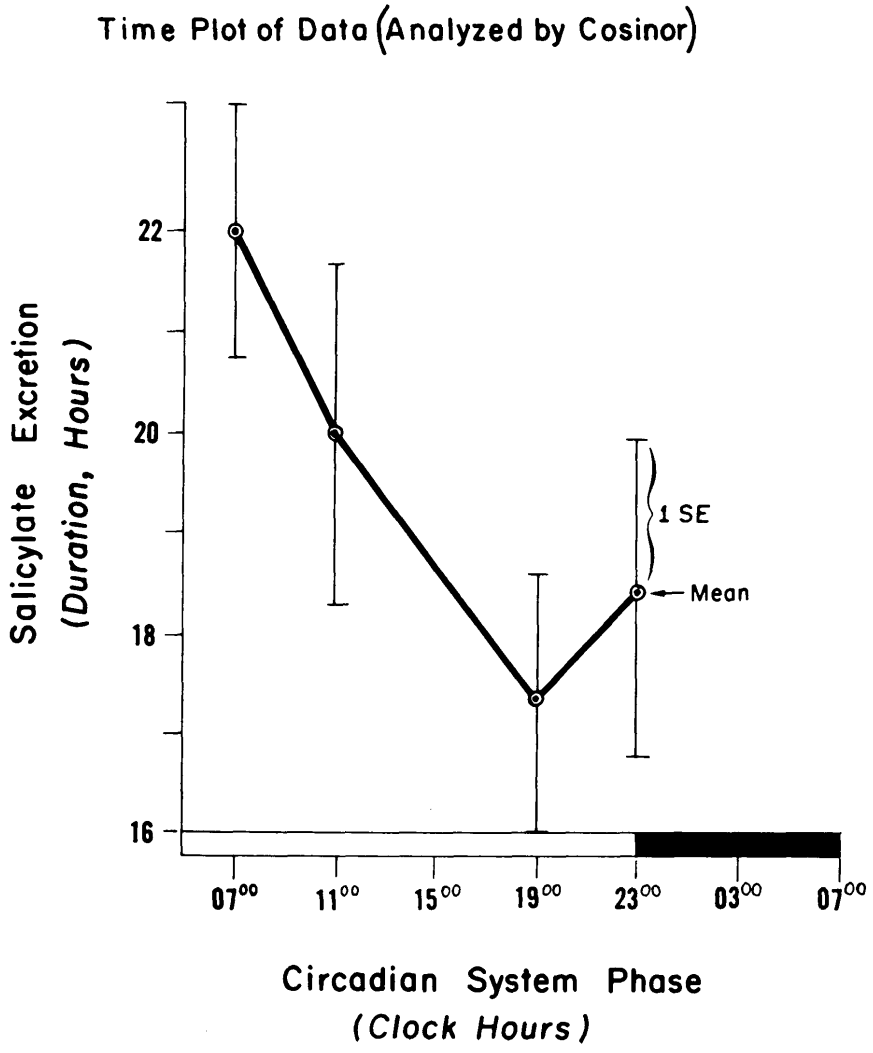
In a cosinor based on the b) series, *i.e.*, on the amounts of salicylate excreted during the first 4 hours after administration of the drug at different circadian system phases, the error ellipse drawn as a 95% confidence region (not shown) overlapped the pole. The limitation of the sample size must be considered in this connection since the b) series are each based upon a single determination in a given test. The a) series, in turn, are based upon more extensive data, namely upon the follow-up of the 4-hourly samples for 48 hours. A cosinor summary of these a) series, at the bottom of Fig. 1, shows, first, a relatively tight error ellipse away from the pole—drawn as a .95 confidence region. This error ellipse does not overlap the pole, even when it is drawn with a confidence coefficient of .998. A circadian rhythm in the excretion of a drug in human beings thus is demonstrated. The same cosinor also indicates the timing and the extent of circadian rhythmic change. Thus, in Fig. 1, the φ, *i.e.*, the angle of the directed line, represents the crest phase, *i.e.*, time of longest duration of salicylate ex-

cretion. The φ is expressed in degrees, with $360° = \tau = 24$ hours and $15° = 1$ hour.

The φ locates the crest at $-100°$ from local midnight (equated to 0°) as shown on top of the scale of the polar plot in Fig. 1. The minus sign before the φ value indicates that φ is expressed as a delay (rather than as an advance) from midnight. Radii drawn tangent to the ellipse delineate the confidence arc of this amplitude-weighted group phase estimate, as discussed elsewhere(7). This confidence arc of the amplitude-weighted φ value extends clockwise from -26 to $-143°$. The timing of the circadian crest, expressed in local time as clock hours and minutes is at 06:41, with a 95% confidence arc from 01:45 to 10:52.

When the middle of the daily span of "darkness" (rest and/or sleep), rather than 00:00, is chosen as phase reference, the φ is $-55°$, the corresponding confidence arc extending clockwise from $-341°$ to $-118°$.

The group amplitude, C, of the circadian rhythm in duration of salicylate excretion is 2.34 hours; the total extent of circadian rhythmic change, *i.e.*, the double amplitude, of course, is 4.68 hours. The 95% confidence interval of the C value extends from 1.44 to 3.24 hours.



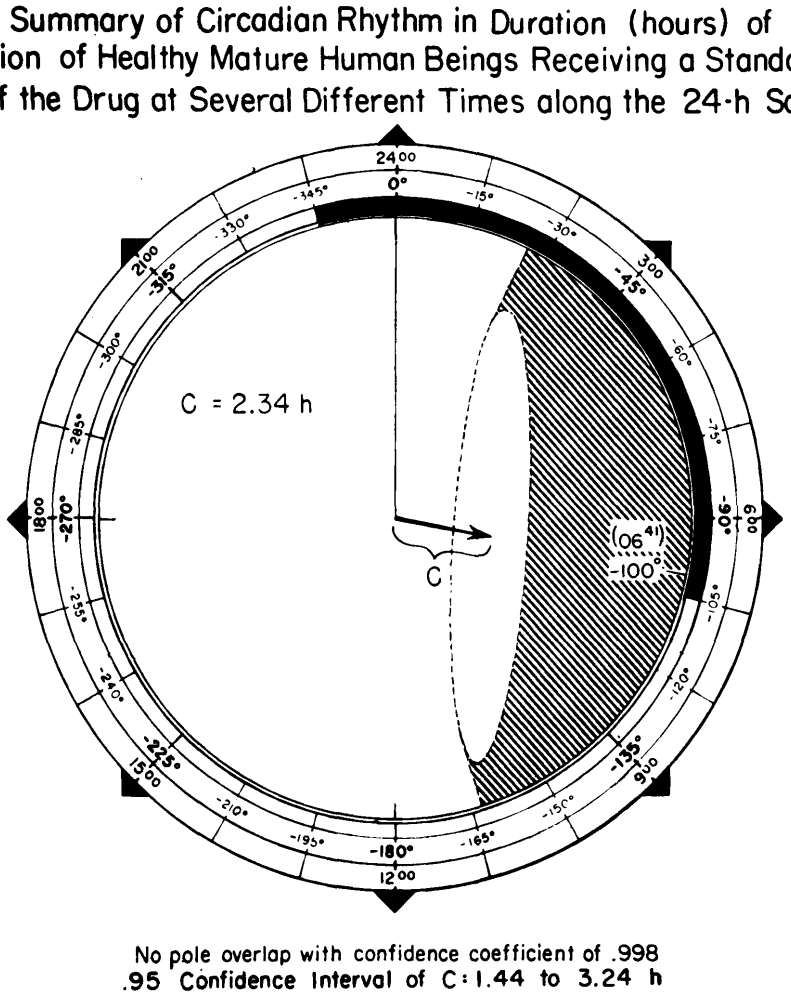


Fig. 1. Plot of time-varying average duration of salicylate excretion on top, analyzed by cosinor; result demonstrates the detection of a pharmacologically interesting circadian rhythm—error elipse away from the pole—and estimation of its parameters, C and φ, by this method (see text).

Fig. 2 presents cosinor analyses of the data from the two circadian-system-phase-reference series on rhythms in 17-OHCS and K excretion summarized in Table I. It can be seen from the top of this Figure that the extent of circadian rhythmic change in 17-OHCS excretion, expressed as percentage of the overall series mean, was 44% in the case of the first profile and 32% in the second. The confidence intervals of these amplitude estimates overlapped. The same applies to the confidence arcs of phase estimates of —191° and —180°.

A similar agreement can be seen for the C and φ estimates of the rhythm in urinary potassium excretion from the cosinor plots at the bottom of Fig. 2.

Discussion. The finding that the duration of the salicylate excretion in man depends upon the circadian system phase at the time when the drug is administered extends the scope of much related evidence obtained

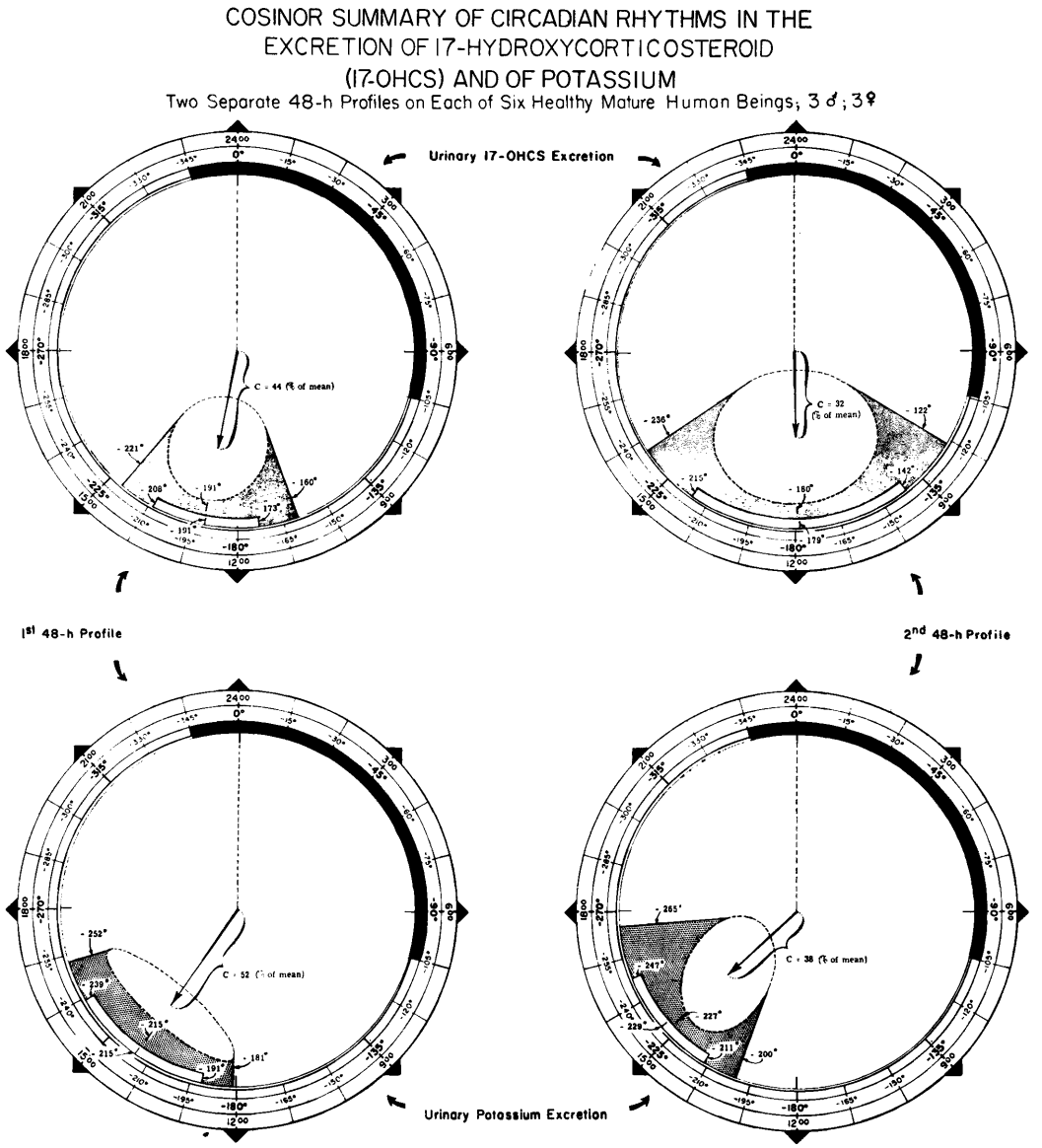


Fig. 2. Reproducibility in 2 circadian-system-phase-reference series (on left and on right of the diagram, respectively)—analyzed by cosinor—of the parameters of rhythms in excretion of 17-OHCS and K. Data in Table I served as computer input. Output φ should be adjusted to synchronizer schedule and may also serve as reference for the pharmacologic rhythm—points made in Table II.

earlier on human beings(3) and experimental animals(13) on certain temporal differences in the biologic response to different agents, including drugs.

These results are reported without a view of practical implications. Obviously, whenever salicylate is taken for analgesia, its timing will usually be determined by the occurrence of pain. Furthermore, to complicate matters, pain itself, more often than not, is an intermittent phenomenon and, on occasion, a rhythmic one. By 1937 Jores indeed reported circadian rhythmic behavior with respect to experimentally-induced pain(14). His original data describe the time course of pain in response to the electrical stimulation of teeth at different clock hours. A recent analysis of these pioneering results(14) carried out in

TABLE I. Relative Changes in 17-Hydroxycorticosteroid (17-OHCS) and Potassium (K) Excretion in Two Separate Series, Each Covering 48 Hours.

Sampling span (day and clock hours)		Values expressed as % of overall mean: 17-OHCS* Series I	17-OHCS* Series II	K* Series I	K* Series II
1	07:00 to 11:00	129 ± 8.7	115 ± 8.4	112 ± 9.8	113 ± 10.4
	11:00 to 15:00	134 ± 14.5	125 ± 10.9	169.7 ± 13	130 ± 11.9
	15:00 to 19:00	123 ± 11.9	118 ± 12.4	122.7 ± 12.8	124 ± 10.9
	19:00 to 23:00	85 ± 6.8	90 ± 8.9	79.7 ± 10.1	104 ± 8.6
1–2	23:00 to 03:00	63 ± 7.5	62 ± 12	61.1 ± 8.7	64 ± 10.6
2	03:00 to 07:00	66 ± 11.9	90 ± 13.4	54.8 ± 5.7	65 ± 5.5
	07:00 to 11:00	137 ± 11.3	130 ± 8.1	102.8 ± 11.3	103 ± 8.4
	11:00 to 15:00	149 ± 19	121 ± 8.9	153.8 ± 19	133 ± 3.6
	15:00 to 19:00	107 ± 14.4	108 ± 8.9	131.5 ± 11.2	122 ± 8.6
	19:00 to 23:00	75 ± 8.6	75 ± 12.1	105.8 ± 7.4	126 ± 11.9
2–3	23:00 to 03:00	55 ± 10.2	51 ± 9.9	58.3 ± 8.7	61 ± 8.9
3	03:00 to 07:00	77 ± 10.4	115 ± 14.9	47.8 ± 8.7	55 ± 6.8

* Mean of relative values (% of overall mean) from 6 subjects and standard errors of each sampling subgroup.
Decimals are of dubious significance.

TABLE II. Circadian Crests of Variables Investigated Expressed in Relation to Different Phase References.

Variable investigated		Phase reference	Crest phase, φ* (.95 confidence arc)
17-OHCS excretion	Series 1	Mid-sleep	−146° (−115 to −176)
	Series 2		−135° (− 77 to −191)
Potassium excretion	Series 1	" "	−170° (−136 to −207)
	Series 2		−182° (−155 to −220)
Duration of salicylate excretion		17-OHCS φ in Series 1	−269° (−195 to −332)
		" " " Series 2	−280° (−206 to −343)
		K φ in Series 1	−245° (−171 to −308)
		" " Series 2	−233° (−159 to −296)

* $\tau = 24$ hr $= 360°$; hence $15° = 1$ hr.

our laboratory with Mr. M. Smolensky, by methods described elsewhere(7), yields a φ value for the crest in pain threshold (least sensitivity) at −122° from local midnight (*i.e.*, at about 08:00) for Jores' presumably diurnally active subjects. The confidence arc of this crest covers the span from −104° to −140°. Among others, Bochnik has studied changes along the 24-hour scale in cutaneous sensibility of human beings(15) and reported further that vasomotor headache exhibits rhythmic changes in its occurrence(16).

Rhythmic changes in the extent of both the desired and the undesired effects from a given dose of a drug remain, of course, the primary determinants of any timing of therapy(5,13), quite apart from the duration of excretion, the sole point examined herein. The statistically significant effect exerted by the circadian system phase upon the duration of salicylate excretion is to be viewed from this much broader viewpoint as merely a minor yet basically interesting facet of the much broader and complex problem of therapy timed as a function of rhythms.

It also should be remembered that circadian rhythms are usually frequency-synchronized with the human subject's long-maintained rest-activity routine and that they also bear a specifiable phase relation to this routine. Thus φ values given in relation to local time have no value whenever subjects live on different synchronizer schedules (5). However, this difficulty can readily be overcome by expressing φ in degrees in relation to midsleep and/or in relation to some other prominent circadian rhythm of the subject (Table II). Such results in degrees

can be compared not only in subjects on different routines but also in subjects on a routine differing from an average 24-hour environmental synchronizer cycle—so long as the prominent frequency of the rhythms compared is the same.

Summary. The duration of urinary salicylate excretion following oral administration of the drug was studied on 6 human subjects standardized on a routine of sleep daily from 23:00 to 07:00. The drug was administered to each of the 6 subjects at 4 different time points, *i.e.*, at 07:00, 11:00, 19:00 or 23:00 (4 separate tests at least 1 week apart). Average duration of drug excretion depended on the time of its administration. A circadian rhythm in this pharmacologic phenomenon was detected and analyzed by the so-called cosinor method and such results agreed with impressions gained from a conventional time plot of the data. By cosinor, duration of salicylate excretion was found to be longest when the drug was administered about 06:41. The 95% confidence arc of this circadian crest-phase estimate extends clockwise from 01:45 to 10:52. When expressed in degrees (with 360° = 24 hours), in relation to the middle of the daily span of rest and/or sleep, the crest φ of the rhythm in drug excretion occurs with a delay of 55° from this φ reference, the confidence arc of the crest extending clockwise from −341° to −118°. The pharmacologic rhythm here reported also is expressed in relation to the timing, in the same subjects, of 2 well-known excretory rhythms—in 17-OHCS and potassium excretion. The latter rhythms were determined on each subject in 2 separate 48-hour profiles and were also evaluated by the cosinor method, with excellent agreement of the φ and the C from one profile to the next.

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