originally learned composition (optical, acoustical, scenic, etc. components) inter alia due to the higher probability of appearance of one element which promotes the retrival of the other components. Addition of new or deviating pattern elements leads to new 'ideas', weakening, distortion or suppression of original impressions, or repressions if the filters are set to block passage of patterns of unpleasant experience. It is seen that neither RNA modification nor loops are required to explain the various phenomena.

In simple language, our thinking may amount to stimulating a 'probability filter system' which then sees to it that the patterns passed on to the conscious mind correspond to our world of experience. We 'tap' the system, feeding in this and that stimulus which we believe is relevant. If the brain has been properly fed with information and was well maintained in the past, it may then supply us with the desired flow of thought, related trains of ideas may be triggered, and when we ultimately try to go to sleep, the brain may refuse to be silenced.

STUDIORUM PROGRESSUS

Timing of Single Daily Meal Influences Relations Among Human Circadian Rhythms in Urinary Cyclic AMP and Hemic Glucagon, Insulin and Iron[1]

F. Goetz, J. Bishop, F. Halberg, R. B. Sothern, R. Brunning, B. Senske, B. Greenberg, D. Minors, P. Stoney, I. D. Smith, G. D. Rosen, D. Cressey, E. Haus and M. Apfelbaum

Department of Medicine and Chronobiology Laboratories, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis (Minnesota 55455, USA); the Department of Laboratories, St. Paul-Ramsey Hospital, St. Paul (Minnesota 55101, USA), and Hospital Bichat, Paris (France), 2 March 1976.

Summary. Relations among circadian rhythms in serum iron, glucagon and insulin and urinary cyclic AMP excretion differ drastically when diurnally active, nocturnally resting human adults consume all daily food for one week as breakfast only and for another week as dinner only – a finding of interest to diverse fields, e.g., for optimizing certain kinds of therapy or for a better utilization of calories.

Introduction. Variables at several levels of biological organization in pigeons and mice have been documented to exhibit a circadian rhythm persisting in the absence of all food and water, until death from starvation and dehydration[2–6]. This circadian rhythm persistence also has been demonstrated by Apfelbaum et al.[6] and Reinberg[7] for obese human subjects on a hypocaloric diet. In the latter studies, relatively minor if not negligible alterations were found in the rhythms' timing when a single 250 calorie meal was consumed at different times.

By contrast, time relations among circadian rhythms in mice could be shown to differ according to the location along the 24-hour scale of a 4-hour span of access to food, a span designed to simulate a substantial yet single daily meal[8].

Moreover, when daily food consumption was restricted to the start of the usual span of activity – 'breakfast only' – nocturnally active mice on a schedule of light alternating with darkness at 12-hour intervals, had a lower body weight than was the case when food was consumed as 'dinner only'; i.e., when mice were allowed access to food only during early light, presumably the equivalent of a very late supper or sleep-interrupting snack. The same finding also applied to human beings. Diurnally active volunteers had a relative body weight loss when they ate 2000 calories per day only in the morning, as compared to a span when they ate the same amount only in the evening[9]. Statistically significant differences in internal relations of human circadian rhythms dependent upon meal-timing were also observed and will be described herein.

Materials and methods. For 2 consecutive 1-week spans, 5 male and 2 female presumably healty human volunteers followed a more or less sedentary routine of wakefulness from 06.30 h to 23.30 h and rest (mostly sleep) during the remainder of each 24-hour span.

All subjects were given a complete physical examination, which did not uncover organic disease; they were interviewed to estimate usual caloric intake, food preferences, and eating times before establishing a meal plan. An average daily level of 2000 calories was determined and used as the basis for a catecholamine-free controlled-nutrient plan. The 2000 calories were distributed as 50% carbohydrate, 15% protein and 35% fat. Although the calculated protein intake remained constant from day to day, amino acid content and food composition did vary. Iron intake was fixed at 13 mg. The total fluid intake from the diets (milk, fruit juice, soft drinks and water) was set at 3,000 ml/day to ensure adequate urine output. The intake of non-caloric drinks was not restricted in time. For 3 subjects breakfast and dinner menus differed in kind, though not in total calories; for the other 4 subjects (including the 3 described in the Figure and Table II), menus were the same during the week on breakfast and that on dinner. The subjects were monitored by the staff of the General Clinical Research Center at the University of Minnesota.

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Table I. Circadian relations among hemic variables altered by timing of single daily meal in 7 presumably healthy volunteers

Variable	No. of Subjects	No. of observations	% Rhythm (P) [a]	Amplitude (SE)	Acrophase (ϕ) [c] ϕ reference: 00.00 h ϕ (95% C.I.) degrees	Clock h min	ϕ reference: meal onset [b] (95% C.I.) degrees
Breakfast-only							
Plasma insulin	7	56	27 < 0.001	75.6 22.0	-156 (-119 -191)	10.24	-51 (-14 -86)
Plasma glucagon	7	56	42 < 0.001	29.8 6.2	-169 (-144 -193)	11.16	-64 (-39 -88)
Serum iron	6	50	46 < 0.001	38.1 7.7	-109 (-86 -133)	07.16	-4 ($+19$ -28)
Dinner-only							
Plasma insulin	7	56	39 < 0.001	100.9 22.5	-299 (-272 -326)	19.56	-37 (0 -54)
Plasma glucagon	7	56	51 < 0.001	33.9 5.9	-16 (-356 -35)	01.04	-114 (-84 -123)
Serum iron	6	52	10 0.075	15.4 —	-240 —	16.00	$+22$ —

[a] Percentage of variability accounted for by cosine curve. [b] Breakfast from 07.00 ($-$ 08.00 h); dinner from 17.30 ($-$ 18.30 h). [c] 360° ≡ 24 h; 15° = 1h; C. I. = Confidence Interval.

Two of the subjects started with 'breakfast only' at 07.00 h and the other 5 with 'dinner only' at 17.30 h, each meal being ingested within 1 h. Subjects ate the entire 2000 calorie meal with but minor deviations. After 1 week on these schedules, the timing of the single daily meal was changed so that those who had been on 'breakfast only' were now on 'dinner only' and vice versa. These new schedules continued for another week. On the last day of each 1-week span blood and urine samples were obtained during a 24-hour span, at intervals varying among subjects from 2 to 4 h.

Total serum iron per 100 ml was determined as described by NELSON[10]. Insulin was measured by double antibody precipitation radioimmunoassay using iodine^{125}-labeled insulin[11]. Glucagon was determined directly on plasma by a radio-immunoassay using Unger's antibody 30K, presumably specific for pancreatic glucagon. Dextran-coated charcoal was used for the separation of bound and free hormone. The sensitivity of the assay is in the range of 20 to 1000 pg per ml. Cyclic adenosine 3′,5′monophosphate (cyclic AMP) was determined in urine by the method of GILMAN[12]. All data series were fitted by least squares with 24-hour cosine curves to test the possibility of zero amplitude (i.e., no rhythm) and, when this hypothesis was rejected, to obtain from the best-fitting curve point and 95% confidence interval estimates of two characteristics, one a measure of extent of change, the amplitude (the distance from the midline to the peak), the other a measure of timing, the acrophase (the lag of the peak from local midnight)[13].

Results. On either meal schedule, the subjects exhibited a prominent circadian group rhythm ($p < 0.01$) in data expressed as percent of mean for serum iron and plasma insulin and glucagon, as summarized in Table I, on the basis of the single cosinor method[13]. Peak values for both plasma glucagon and insulin are found after the consumption of the meal, although the relation of the peak in glucagon to the single meal is different on the regimen of 'breakfast only' as compared to that on 'dinner only' ($p < 0.05$). Peak values for serum iron are found in blood withdrawn near or before the time of eating on both meal schedules. The time course along the 24-hour scale, including the post-prandial drop of serum iron, differs after breakfast as compared to dinner, whatever the unknown underlying factors may be.

On 'breakfast only', glucagon and insulin show a close time relation to one another. Since 360° ≡ 24 h and

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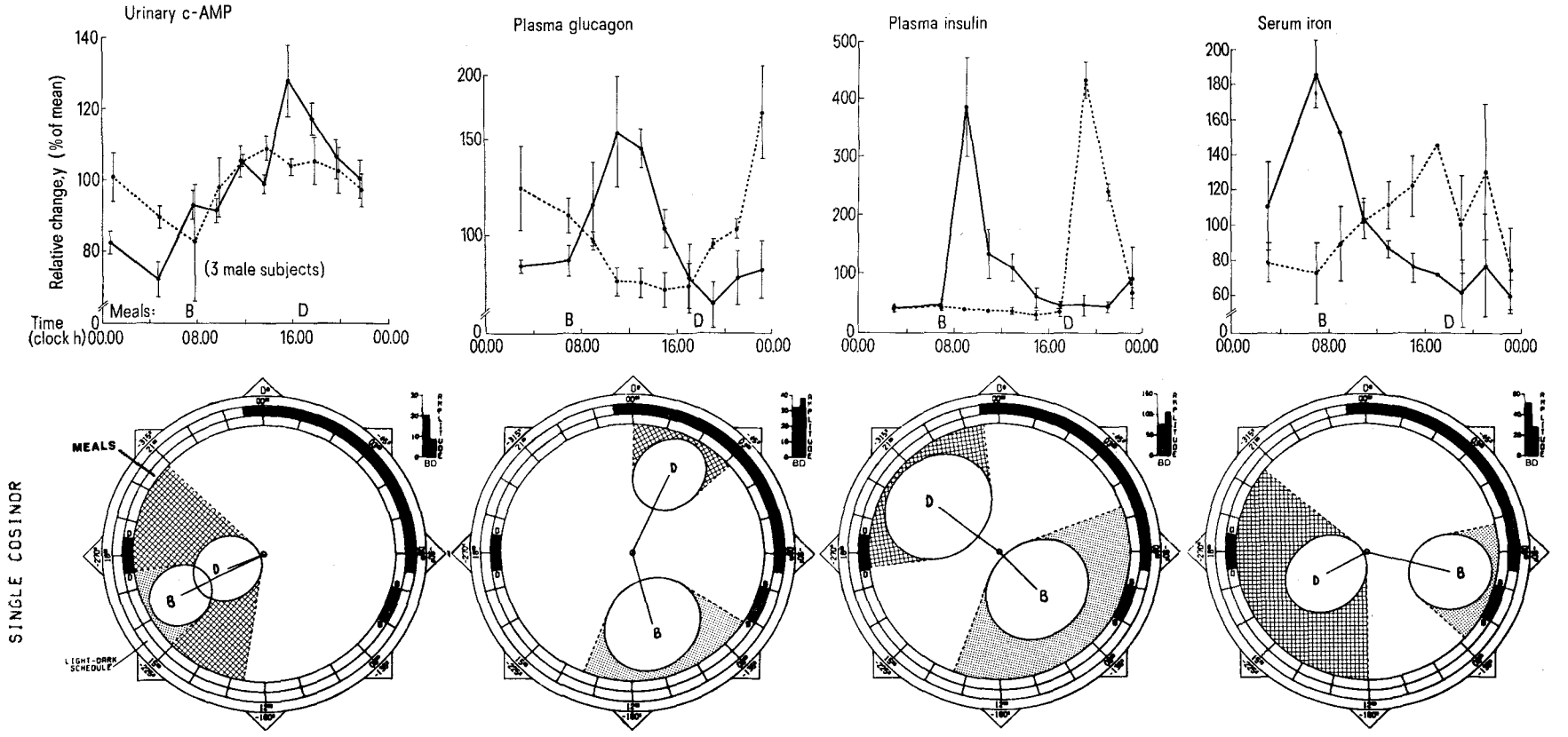
Table II. Circadian rhythm in urinary cyclic AMP excretion by 3 presumably healthy men consuming single daily meal as breakfast or dinner

Subject (years of age)	No. of observations (% Rhythm) [a]	Mesor (SE) nM/hr	Amplitude (SE)	Acrophase (ϕ) [d] ϕ reference: 00.00 h degrees	Clock h min	degrees
Breakfast-only (07.00–08.00 h)						
1 (24)	27 (71) [c]	207.9 (3.3)	36.2 (4.7)	-251	16.44	(-238 -265)
2 (21)	28 (39) [c]	241.8 (8.7)	49.0 (12.7)	-233	15.32	(-207 -259)
3 (19)	19 (66) [c]	290.8 (8.0)	65.3 (11.7)	-237	15.48	(-219 -255)
Dinner-only (17.30–18.30 h)						
1 (24)	28 (40) [c]	214.9 (4.2)	24.4 (6.0)	-190	12.40	(-164 -216)
2 (21)	18 (35) [b]	218.2 (5.7)	24.3 (8.5)	-224	14.56	(-192 -256)
3 (19)	18 (36) [b]	281.5 (7.8)	30.6 (10.6)	-290	14.20	(-249 -330)

[a] % Rhythm, percentage of variability accounted for by cosine curve; [b] $p \leqslant 0.05$; [c] $p \leqslant 0.01$. [d] 360° ≡ 24 h; 15° = 1 h



Circadian rhythms differently influenced by a single daily 2000 calorie meal given for 7-day spans.
Blood and urine samples were obtained every 2 h during waking and once in the middle of the sleep-span from each of 3 young, male volunteers during the last 24 h of one 7-day span of eating breakfast-only (B) and another 7-day span of eating dinner-only (D). Each data series on plasma glucagon, insulin, serum iron and urinary C-AMP (*nM*/h) was converted to % of the respective 24-hour mean. In the chronograms (top row) the means and SE of these converted values across all 3 subjects are plotted against the sampling time, each chronogram presenting results for a given variable on B (—) and D (---). The cosinor plots (bottom rows) depict results of fitting a single 24-hour cosine curve to all data on each variable and meal schedule. The length and direction of each radial line (vector) indicate the amplitude and acrophase, respectively, of the fitted cosine. The ellipse at the tip of each vector represents its 95% confidence region. Statistically significant rhythms in all variables are indicated by the fact that none of these confidence regions overlaps the 'pole' (center of plot). Whereas the timing of insulin, glucagon and iron rhythms are different on B and D, that of C-AMP, as a group phenomenon, is similar on the 2 meal schedules (for interindividual differences, see Table II).

hence 15° = 1 h, point estimates for the acrophases of the insulin and glucagon rhythms, at −156° and −169°, correspond to 10.24 h and 11.16 h, respectively. This difference of 13° is less than 1 h and the confidence intervals overlap widely; no significance is attached to any difference in timing of rhythms in these two variables on the regimen of breakfast only. The serum iron acrophase at −109° leads the acrophase for glucagon, without overlap between the 95% confidence intervals of these two variables – a finding denoting conservatively a statistically significant difference in timing.

On 'dinner only', point estimates for the acrophase of rhythms in glucagon and insulin of plasma were 77° (over 5 h) apart, and, as can be seen in Table I, their 95% confidence intervals did not overlap. This non-overlap documents the statistical significance of a difference in internal timing of glucagon and other rhythms in subjects on the 'dinner only' regimen, whereas no such difference is seen in the 'breakfast only' group. The finding as a whole extends to human beings the earlier results obtained on other variables of the mouse[8] demonstrating that the internal timing of rhythms can be manipulated by the timing of meals.

The Figure shows a statistically significant circadian rhythm in urinary cyclic AMP excretion, a variable examined thus far in the urines of only 3 male subjects of this study. Results on other variables, for these 3 subjects only, also are demonstrated in this figure. On the average, the rhythm in cyclic AMP excretion had a similar timing on breakfast and on dinner although individual subjects showed differences in timing, Table II. As to the effect of meal timing upon the extent of urinary cyclic AMP excretion in the 3 subjects examined, the amplitude of the circadian rhythm was higher on breakfast as compared to dinner, Table II. All in all, the grouped or the individual changes in the timing of the circadian rhythm in urinary cyclic AMP excretions, in the small sample of 3 subjects examined, did not correspond to the much more extensive change in the timing of rhythms in glucagon, insulin or iron of blood.

Discussion. The statistically significant difference in internal timing of glucagon and insulin on 'dinner only', as compared to the state on 'breakfast only', may contribute to the relative loss in body weight on 'breakfast only'[9]. However, differences in overall body activity have not been ruled out as a factor underlying such results. One may be more active on a regimen of 'breakfast only' than on a regimen of 'dinner only'. On the other hand, motor or other activity may be associated with a different caloric 'cost' at different circadian times, so that a relative body weight loss may occur when a given amount is consumed at one predictable time as compared to another even if total body activity were identical or at least similar, as was likely the case for the subjects here examined on breakfast only.

As early as 1814 VIREY[14] wrote of a 'different temperament' as a function of whether one eats a single daily meal in the morning or in the evening. The hormonal findings here quantitatively documented are also consistent with the possibility that the metabolic fate of a meal will differ at different circadian (meal) times, inter alia, because of widely differing hormonal relations, quite apart from any differences in extent or timing of motor activity. The findings should be viewed in the context of suggestions by Potter, Ehret and others[15–17] that meals as well as drugs be manipulated in time in various studies of rhythms. Those interested theoretically in the post-prandial kinetics of insulin, glucagon or iron in human blood can anticipate circadian-state-dependent responses to be viewed against reports of 'meal-induced' rhythms in the synthesis of hepatic enzymes. In any event, circadian chronokinetics also are of applied interest – for manipulating the relations among hormonal rhythms in order to optimize the utilization of food or drugs[17].

Changes in internal circadian timing following rescheduling of meals in the LOU rat carrying a transplanted immunocytoma coincided with an overall improvement in results of chronotherapy with adriamycin[18,19], but the responsible rhythmic factors remain to be elucidated. The possibility remains that circadian rhythms in serum[18–20] or urinary iron[21], among other variables such as polyamines, may serve as markers guiding chronotherapy in human beings and rats[18–20].

In connection with the rhythm in serum iron, possibly rhythmic increases in the binding of iron to transferrin come to mind. Circadian variation in plasma iron turnover has been noted by LOCKNER[22] who sampled twice on each of his subjects, between 18.00 h and 20.00 h (1 or 2 h after dinner) and on the following morning, after fasting. Hence his data are not comparable. Our results certainly extend the scope of earlier work demonstrating that the serum iron rhythm persists in presumably healthy volunteers consuming equidistant isocaloric meals with equal iron content[23]. This fact notwithstanding, it is important to control meal time in studies on serum iron. One must search for possible differences, i.a., in meal schedules when the timing of prominent circadian rhythms in serum iron varies greatly among individuals, as documented by WERNER and GLADTKE[24], in the context of kinetic studies.

Circadian variation in urinary cyclic AMP has been reported earlier[25–27] and may reflect changes in cyclic AMP of muscle. It will be interesting to study variations in this variable at the sites of hormone action. Thereby new means of analyzing (and ruling out) obligatory mediators are available. Thus, to the extent that any rhythms in cyclic AMP in blood and at the cellular sites of hormone action behave similarly to the time course of cyclic AMP in urine, and to the extent that any such rhythms in cyclic AMP may shift only slightly or not at all, following a change from breakfast to dinner, a degree of independence of cyclic AMP mediation from the hormone effect is established. The interest in these studies stands from the important differences in body weight change on ‚breakfast only' as compared to 'dinner only'[28].

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