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CIRCADIAN RHYTHMS IN N.Z.W. RABBITS UNDER DIFFERENT FEEDING SCHEDULES *

Rosi F., Greppi G.F., Corti M., Nordio C.

Cattedra di Anatomia e Fisiologia, Istituto di Zootecnia Generale, Facoltà di Agraria, Università degli Studi, via Celoria-2, 20133 - MILANO (Italia)

INTRODUCTION

Persistent daily fluctuations occur in a large number of physiological variables of the living organism. These rhythms in animals are necessarily reflected in chemical bioassays, because values of some measured functions vary from hour to hour over the total time span of the rhythm(Altman and Dittmer,1973;Scheving and Pauly,1974).Many blood parameters used to assess the nutritional status of animals are subject to circadian fluctuations. These fluctuations are related to variations of feed consumption schedules, diet composition, lighting cycle, environmental temperature, light intensity and athmospheric pressure(factors known to affect the animal metabolism)(Aschoff, 1965; Reinberg,1974). The investigation reported is part of a research devoted to study the rabbit time structure, and mainly its relationship with nutrient metabolism. Because of the influence of digestion and absorption kinetics and related metabolic pathways upon blood parameters daily fluctuations, the purpose of this study was to investigate the influence of feeding schedules, when other factors were standardized.

MATERIAL AND METHODS

24 male adult inbred N.Z.W. rabbits of the same age, weighting about 3 kg were used. They were housed in individual stainless steel cages under a lighting cycle from 8 to 20 h. The animals were divided in 3 omogeneous groups fed a commercial diet: the A group had continuous feed access(ad libitum fed); the B group had feed access restricted to 8-20 h span(light-fed); the C group to 20-8 h span(night-fed). After a 30 days on these feeding regimens, the 24 rabbits were bled and body temperature recorded at selected times(4,8,12,16,20 and 24 hours)over a 21-days period, at prefixed dates following a rotation program(as per Rosi and coll.,1981), always leaving an interval of at least 90 hours between 2 subsequent bleedings on the same animal. Blood samples (about 6 ml) were taken from ear marginal vein. The 144 sample were immediately tested for hemoglobin level and the obtained serouses were frozen until tested for levels of glucose,total lipid, cholesterol, triglyceride, total protein,transferrin,urea, calcium,iron, inorganic phosphorus and magnesium and for proteic fractions percenta

ges(following analytical procedures reported in Rosi and coll.,1981).Rectal temperature was recorded before every bleeding. Individual daily feed consumption and weekly weight gain were also recorded.The time series pertaining to each animal for each investigated physiological variable were processed by statistical method of periodic regression(Halberg's cosinor test) to evaluate the evidence of circadian periodicity and to estimate the parameters of cosine curves fitted to the data. The F statistic was used to test the zero amplitude hypothesis for determining whether or not the data can be described by a cosine function at 0.05 probability level. The cosine functions fitted to the data were $y = \bar{y} + A\cos(\omega t + \varphi)$ where $\bar{y}$= mean level of the physiological variable, A= amplitude,i.e.half extent of a rhythmic change in a cycle, $\omega = 360°/24\ h = 15°/h$= angular velocity, t= hour of the day(independent variable), φ = acrophase,i.e. time expressed as degrees, when the cosine function attains its maximum. For each physiological variable the daily pattern of the 3 experimental groups was also compared to state the influence of different feeding regimen on mean levels and on circadian biorhythms.

RESULTS

No significant differences among the 3 experimental groups were found, when individual weight gain was related to initial body weight and feed intake.Neither slope nor elevation of the regression line were affected by the feeding schedules. Furthermore, no statistically significant differences were found among the 3 groups in daily feed intake either. The animals under different feeding schedules showed different mean levels of total protein, albumin,hemoglobin,urea and Fe. The total protein,albumin and Hb concentration of A group(ad lib-fed group) layed in between of those of B and C groups, while mean level of urea and of Fe was lower in A group. Dark-fed animals (C group) showed higher mean concentrations of these 5 blood constituents. For each tested variable results concerning cosine curves,fitted to the data by cosinor test, and and their statistical significance are summarized in tab.1(data pertaining to ensamble of the 24 rabbits of the 3 groups),tab.2(group A),tab.3(group B) and tab.4(group C), where $\bar{y}$ is daily mean concentration, A amplitude, s standard deviation, r amplitude 95% confidence interval,(all expressed as indicated units), φ acrophase, IC acrophase 95% confidence interval,(both expressed as hours and minutes) and F the statistical significance. The cosine curves describing the pattern of a variable as related with hour of the day are plotted, and fig.1,2 and 3 are some examples. These graphs show for each group the daily pattern of rectal temperature(fig.1),total lipid(fig.2) and

Fe(fig.3) levels, as functions of the time(abscissa). The mean level of each experimental group is also indicated.

DISCUSSION

The physiological variables investigated in this study can be divided in those that vary rhythmically during the 24 h period and variables that do not. In the first category we find 1) parameters whose circadian fluctuations are in phase accordance regardless the feeding schedules, i.e. rectal temperature, Hb and α_2-globulin levels. Since the oscillations of these variables appear independent from feed intake time, it can be assumed they are endogenous and merely synchronized with the environmental photoperiod cycle. In present study the body temperature peaks during the darkness (fig.1). Our findings are consistent with observations performed on many animal species, showing that the oscillations of body temperature reach the highest values during the activity period of the day and that this rhythm is hardly shifted or disrupted(Turek, 1981; Carandente and coll., 1982). Our results indicate also that similar cyclical patterns in Hb level occur in rabbits under different regulated feeding schedules and that the cosine functions exhibit their maximum during the light span of the day. These observed diurnal variations agree well with those reported by Fox and Laird(1970a) on rabbit. In man this parameter is known to vary on a diurnal pattern with highest levels during the period of activity. Regarding time of the day our data agree with those of human. However, when compared on the basis of activity cycles, assuming the rabbit is a nocturnal animal, the data do not correlate. It has been argued that in man the exercise brings about a release of erytrocytes due to oxigen debt into the circulation by mean of splenic contraction, raising the level of circulating Hb. However, with respect to this point no conclusive explaination can be here presented on the basis of these results. The α_2-globulin level also shows significant biorhythm with acrophase during the first hours of the darkness, regardless the dietary regimen. These findings agree with data obtained from rabbits fed ad libitum as reported in a previous paper(Rosi and coll., 1981); since the main α_2-globulin function is in lipid transport, these circadian oscillations are to be abscribed to lipid absorption and metabolism and to daily pattern of hepatic protein synthesis. 2) variables showing biorhythmic pattern when the data pertaining the 24 rabbits are pooled, but also showing diurnal pattern of oscillations with phase angle differences in animals having different eating schedules, i.e., Ca and total lipid. Recent researches performed on rat have advanced the concept that the circadian rhythm for the Ca level is endogenous in nature and modulated by calcitonin secretion cy-

clical pattern, one of the functions of this hormone being to prevent post-prandial hypercalcemia, thus Ca absorbed from gastrointestinal tract would be preserved for the skeleton(Staub and coll.,1979;Hirsch and Hagaman,1982). These rhythms were not influenced by an abrupt change in feeding habits, but nothing is said about prolonged change. Since in our rabbits the maximum values of Ca levels occurred at the hours immediately following the end of feeding period(as in rat), we presume the diurnal rhythms to be endogenous and mediated by some endocrine clock, and that 2 months of regulated feeding schedules induced an adaptive response(even if incomplete) to new feeding conditions. Analogous mechanism can be supposed in total lipid cyclic variations(fig.2).

3)variables showing circadian pattern of oscillation cued by feeding schedules, i.e. cholesterol, triglyceride, urea, Fe, in. P, and Mg. Since the light-fed group shows biorhythms with angle phase difference of 12 hours in comparison with those of the group fed ad lib and the group dark-fed, it can be postulated these oscillations are passive systems driven by feeding timing. The urea biorhythm which alone peaks during the feeding period, is in agreement with data reported by Fox and Laird(1970b). Concerning the comparison among the acrophases of these blood constituents, it can be noticed that every group displays highest values(with the exception of urea) in the hours following the feeding period, during the post-absorptive state. Our findings do not evidence circadian pattern for levels of a second category of parameters, i.e., glucose, total protein, albumin, some globulin fraction and transferrin, differently from what resulted in our previous study (Rosi and coll.,1981) on male rabbits fed ad libitum. This failure can be probably attributed to reduced number of animals per group attained in this experiment, as interindividual variation can obscure a collective biorhythmic phenomenon, or to interference of caecotrophy, normally practised in the early morning by rabbits fed ad libitum and repeated by 20-30% of animals during the afternoon(Jilge, 1982), or to disturb of caecotrophic activity due to regulated feeding schedules. Anyhow, highest values of proteic blood constituents were recorded in all the 3 groups (apart from some exception in A and C group) during darkness, accordingly with our data and with data reported on rabbit (Fox and Laird,1970b). We can also observe that the C group fluctuations of variables are in phase accordance with those of A group and that there is a 12 h phase difference when compared with those of B group. This should not surprise as subsequent observations revealed that ad lib-fed adult male rabbits whose feed was renewed at 20 h intook 65-75% of daily consumption during darkness, mostly during the first 4-8 hours.

The data as presented would indicate clearly the necessity of considering the time structure of an experimental animal in any investigation involving bio-assay or in studies on the mechanism of nutrient metabolism. Food may be managed differently when given in different phases of the circadian physiological system.

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SUMMARY

Metabolic processes induced by feeding are not steady as they vary depending both on quality of feed and on meal timing. These variations are due to differences in the metabolic pathways during the 24 h span. The aim of this study is to analyze the influence of different feeding schedules along the 24 hours scale on blood parameters and body temperature. From 24 adult male New Zealand White rabbits(nocturnally active animals) reared in controlled conditions with a lighting period from 8 to 20 h and divided in 3 groups(group A:ad libitum feed access;group B:feed access restricted to 8-20 h span; group C:feed access restricted to dark span) blood samples from ear marginal vein were collected and body temperature was recorded by a rotation program at 6 different time(4,8,12,16,20 and 24 h). From this study can be concluded that the biorhythms of b.temperature, Hb and α2-globulin levels are not influenced by the feeding regimen and that the circadian fluctuations of Ca and tot.lipid are only slightly affected, indicating they are endogenous rhythms synchronized by light/dark cycle or depend on some endocrine clock,adjustable by feeding factors. The fact that the shift in the biorhythms of cholesterol, triglyceride, urea,Fe,Mg, Pi levels are closely related to the feeding shift of 12 h suggests that the feeding itself is an important cue for this diurnal variation. No biorhythms are found in glucose,tot.protein, some electrophoretic fractions and transferrin levels.

RIASSUNTO

Il presente studio è stato condotto su 24 conigli maschi N.Z.W., allevati in condizioni controllate e suddivisi in 3 gruppi(gruppo A: alimentato ad libitum;gruppo B; alimentato di giorno; gruppo C:alimentato di notte). Il periodo di illuminazione era dalle 8 alle 20. Secondo un programma a rotazione a 6 ore del giorno prefissate in giorni prestabiliti,agli animali venivano prelevati alcuni ml di sangue dalla vena marginale dell'orecchio e registrata la temperatura corporea. Sono state misurate le concentrazioni di alcuni componenti dei campioni ematici. Dall'analisi dei dati si può concludere che mentre i bioritmi della temperatura e delle concentrazioni di Hb e α2-globuline non sono influenzati dal diverso orario di somministrazione degli alimenti e i ritmi delle concentrazioni di Ca e lipidi totali lo sono in qualche misura, quelli della concentrazione di colesterolo, trigliceridi,urea,Fe,Mg e P sono strettamente dipendenti dall'orario di somministrazione degli alimenti. Le variazioni giornaliere di concentrazione ematica di proteine totali,transferrine, di albumina, di glucosio e in generale delle globuline nel presente lavoro non sono risultate cicliche.

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Tab 1 TOTAL

PARAMETERS	$\bar{y}$	± s	A	± r	φ	IC	F
TEMPERATURE °C	39.55	0.21	0.202	0.06	0^{26}	1^{07}	**
HEMOGLOBIN g/100ml	12.89	1.06	0.31	0.307	12^{35}	2^{59}	*
GLUCOSE mg/100ml	141.68	14.32	1.36	4.14	1^{12}	4^{47}	ns
TOTAL LIPID mg/100ml	277.91	91.63	28.39	26.51	8^{28}	2^{52}	*
TRIGLYCERIDE mg/100ml	114.66	48.48	10.37	14.02	8^{22}	3^{34}	ns
CHOLESTEROL mg/100ml	32.22	6.43	1.36	1.86	5^{49}	3^{35}	ns
TOT. PROTEIN g/100ml	6.4	0.44	0.035	0.126	22^{05}	4^{56}	ns
ALBUMIN g/100ml	4.21	0.36	0.018	0.103	10^{17}	5^{21}	ns
α_1 -GLOBULIN g/100ml	0.47	0.072	0.006	0.021	23^{55}	4^{54}	ns
α_2 -GLOBULIN g/100ml	0.33	0.095	0.024	0.024	20^{39}	3^{01}	*
β -GLOBULIN g/100ml	1.01	0.295	0.053	0.084	21^{03}	3^{52}	ns
γ -GLOBULIN g/100ml	0.38	0.125	0.027	0.032	7^{00}	3^{22}	ns
UREA mg/100ml	40.05	8.39	2.18	2.33	21^{28}	3^{08}	ns
CALCIUM mg/100ml	15.89	0.91	0.27	0.25	16^{41}	2^{53}	*
PHOSPHORUS IN. mg/100ml	4.92	0.98	0.1	0.27	16^{58}	4^{37}	ns
MAGNESIUM mg/100ml	2.78	0.37	0.066	0.107	10^{00}	3^{53}	ns
IRON µg/100ml	237.67	65.57	17.35	18.92	17^{12}	3^{10}	ns
TRANSFERRIN mg/100ml	368.17	60.26	6.28	16.56	6^{23}	4^{37}	ns

Tab 2 GROUP A

PARAMETERS	$\bar{y}$	± s	A	± r	φ	IC	F
TEMPERATURE °C	39.57	0.52	0.165	0.11	0^{15}	2^{13}	**
HEMOGLOBIN g/100ml	13.06	1.14	0.385	0.62	10^{16}	3^{53}	ns
GLUCOSE mg/100ml	140.53	10.01	3.43	5.21	9^{40}	3^{46}	ns
TOTAL LIPID mg/100ml	270.8	94.67	45.93	46.28	10^{16}	3^{01}	*
TRIGLYCERIDE mg/100ml	93.69	44.82	17.135	22.77	11^{03}	3^{32}	ns
CHOLESTEROL mg/100ml	28.82	5.44	3.34	2.83	8^{48}	2^{41}	*
TOT. PROTEIN g/100ml	6.42	0.47	0.03	0.247	0^{05}	5^{32}	ns
ALBUMIN g/100ml	4.22	0.37	0.06	0.183	1^{26}	4^{47}	ns
α_1 -GLOBULIN g/100ml	0.44	0.06	0.013	0.033	16^{28}	4^{35}	ns
α_2 -GLOBULIN g/100ml	0.34	0.12	0.02	0.057	16^{29}	4^{43}	ns
β -GLOBULIN g/100ml	1.03	0.28	0.05	0.129	21^{07}	4^{35}	ns
γ -GLOBULIN g/100ml	0.4	0.09	0.053	0.049	10^{07}	2^{49}	*
UREA mg/100ml	37.22	7.56	3.59	3.71	23^{40}	3^{03}	*
CALCIUM mg/100ml	15.88	0.87	0.49	0.42	15^{38}	2^{42}	*
PHOSPHORUS IN. mg/100ml	4.87	0.64	0.38	0.335	8^{43}	2^{44}	*
MAGNESIUM mg/100ml	2.88	0.33	0.11	0.17	12^{28}	3^{49}	ns
IRON µg/100ml	222.44	68.9	50.46	31	15^{50}	2^{06}	**
TRANSFERRIN mg/100ml	355.49	62.1	46.38	27.73	6^{27}	2^{03}	**

Tab 3 GROUP B

PARAMETERS	$\bar{y}$	± s	A	± r	φ	IC	F
TEMPERATURE °C	39.2	0.19	0.195	0.11	23^{50}	1^{53}	**
HEMOGLOBIN g/100ml	12.3	1.53	0.34	0.8	13^{19}	4^{27}	ns
GLUCOSE mg/100ml	136.2	12.44	3.27	6.45	22^{56}	4^{12}	ns
TOTAL LIPID mg/100ml	250.94	104.12	68.03	54.18	3^{22}	2^{34}	**
TRIGLYCERIDE mg/100ml	118.52	61.32	34.19	31.91	1^{22}	2^{52}	**
CHOLESTEROL mg/100ml	35.59	7.37	5.62	3.83	1^{39}	2^{17}	**
TOT. PROTEIN g/100ml	6.1	0.48	0.1	0.24	1^{32}	4^{27}	ns
ALBUMIN g/100ml	3.95	0.39	0.028	0.19	5^{09}	5^{27}	ns
α_1 -GLOBULIN g/100ml	0.49	0.07	0.031	0.037	5^{10}	3^{20}	ns
α_2 -GLOBULIN g/100ml	0.3	0.07	0.052	0.035	20^{59}	2^{16}	**
β -GLOBULIN g/100ml	0.98	0.35	0.067	0.184	22^{11}	4^{40}	ns
γ -GLOBULIN g/100ml	0.36	0.14	0.052	0.065	5^{08}	3^{26}	ns
UREA mg/100ml	41.25	8.56	6.03	4.27	15^{57}	2^{21}	**
CALCIUM mg/100ml	15.98	0.99	0.72	0.43	19^{14}	2^{02}	**
PHOSPHORUS IN. mg/100ml	5.01	0.72	0.775	0.37	3^{13}	1^{43}	**
MAGNESIUM mg/100ml	2.72	0.42	0.16	0.22	24^{50}	3^{37}	ns
IRON µg/100ml	237.34	64.67	40.78	30.49	4^{49}	2^{27}	**
TRANSFERRIN mg/100ml	370.32	53.27	7.89	27.72	19^{18}	4^{56}	ns

Tab 4 GROUP C

PARAMETERS	$\bar{y}$	± s	A	± r	φ	IC	F
TEMPERATURE °C	39.53	0.21	0.26	0.11	1^{15}	1^{35}	*
HEMOGLOBIN g/100ml	13.31	0.93	0.32	0.48	14^{30}	3^{44}	ns
GLUCOSE mg/100ml	148.29	18.8	3.56	9.78	0^{13}	4^{40}	ns
TOTAL LIPID mg/100ml	312	67.75	53.45	35.25	12^{20}	2^{42}	**
TRIGLYCERIDE mg/100ml	131.77	23.93	34.69	12.45	10^{59}	1^{19}	**
CHOLESTEROL mg/100ml	32.25	4.04	1.74	2.1	14^{06}	2^{29}	**
TOT. PROTEIN g/100ml	6.69	0.37	0.098	0.19	16^{46}	4^{12}	ns
ALBUMIN g/100ml	4.45	0.4	0.113	0.184	12^{54}	3^{53}	ns
α_1 -GLOBULIN g/100ml	0.48	0.08	0.025	0.04	20^{49}	3^{51}	ns
α_2 -GLOBULIN g/100ml	0.34	0.2	0.016	0.033	0^{13}	4^{16}	ns
β -GLOBULIN g/100ml	1.03	0.245	0.049	0.127	19^{24}	4^{36}	ns
γ -GLOBULIN g/100ml	0.38	0.112	0.013	0.058	0^{23}	5^{11}	ns
UREA mg/100ml	41.68	6.3	4.86	3.26	1^{11}	2^{16}	**
CALCIUM mg/100ml	15.82	0.75	0.396	0.393	8^{12}	2^{59}	*
PHOSPHORUS IN. mg/100ml	4.89	0.665	1.22	0.346	16^{53}	1^{03}	**
MAGNESIUM mg/100ml	2.73	0.29	0.232	0.151	10^{40}	2^{13}	**
IRON µg/100ml	253.21	60.59	47.39	26.54	18^{19}	1^{57}	**
TRANSFERRIN mg/100ml	378.69	58.17	19.9	27.86	18^{11}	3^{38}	ns

FIG 1

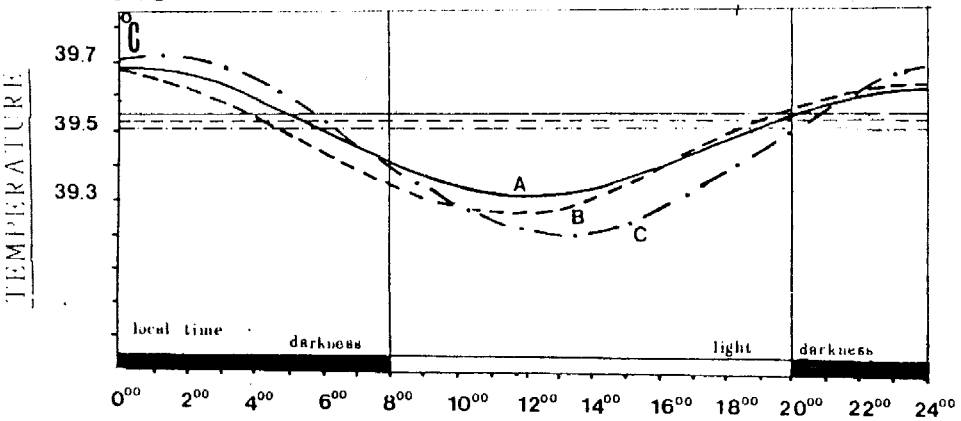


FIG 2

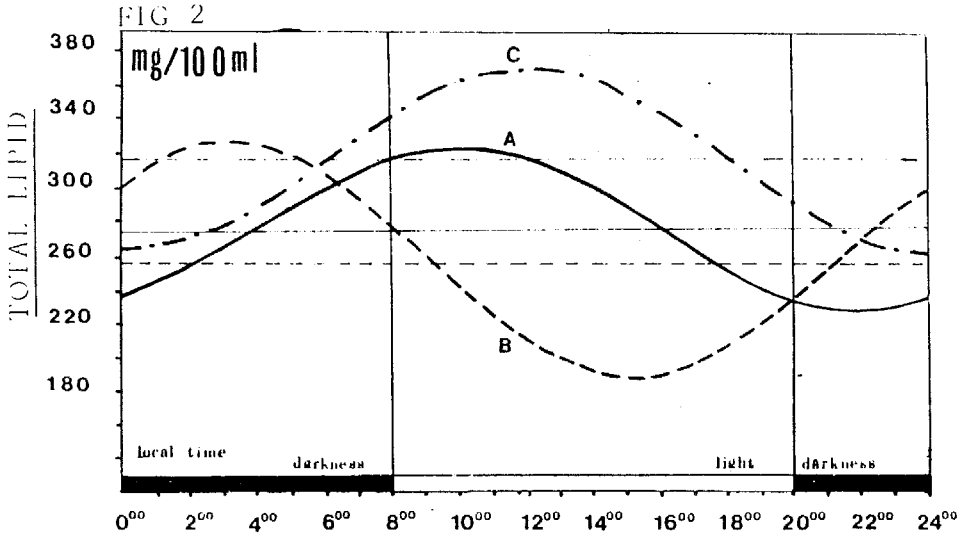
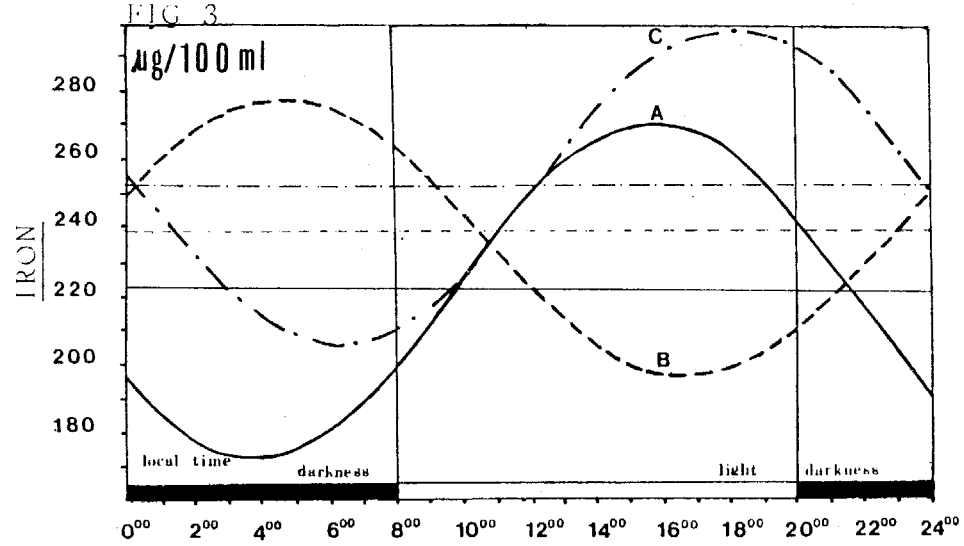


FIG 3



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