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Analysis of Rapid Oscillations of Glucose and Free Fatty Acids in Plasma

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Summary. The authors analyzed rapid oscillations of blood sugar (GL) and free fatty acid levels (FFA) in serum of healthy subjects. They investigated a series of blood samples taken under conditions of absolute rest from the cubital vein at 15-s intervals for a period of 6 min. In addition to common statistical parameters, they calculated the course of autocorrelation and cross-correlation functions and periodograms. The magnitude of oscillations is significantly higher than the error of the biochemical methods. In some sequences periodicities were detected which were statistically significant in 23.8% of GL and in 38.1% of FFA. 24-point series of GL collected in parallel from both arms correlate in 36.3% positively, in 27.3% negatively, and in 36.4% they do not correlate. Series of FFA and GL collected simultaneously from one site correlate mutually in almost all instances either positively or negatively, frequently with a time shift. The oscillations may be due to (a) feedback regulations of the levels of the two metabolites, (b) permanent mutual interaction between the FFA and glucose level and (c) an uneven concentration of the two metabolites in different parts of the circulation. The above factors may combine, and the list of possible factors may not be complete.

Key words: Oscillations of blood sugar level – Oscillations of free fatty acid levels – Harmonic analysis – Periodogram in biochemistry

For some years rapid oscillations of physiological parameters, e.g., blood pressure, heart rate, etc. have been studied (Symposium, 1977). Much less is known about similar oscillations of biochemical parameters, such as the concentration of various substances in blood and other body fluids. Oscillations with intervals of several seconds to minutes have been described in repeated collections of samples for blood sugar levels – GL (Hansen, 1923; Iberall et al., 1968) and of free fatty acids – FFA (Court et al., 1971). Oscillations have also been found in tissue and cell enzyme activities (Ghosh and Chance, 1964). Oscillations of blood levels of GL and FFA, however, have yet to be subjected to detailed mathematical analysis.

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It is important to answer several questions: (a) It is essential to confirm that oscillations of GL and FFA actually exist and are not due to a methodical error. (b) It is important to prove whether oscillations have or have not a periodic character and to assess the length of their period. (c) It must be assessed whether GL and FFA, the levels of which have as a rule an inverse relationship, influence each other also in the course of short-term oscillations. (d) It is important to consider mechanisms which may lead to the development of oscillations in the levels of the investigated metabolites.

The basis of the analysis is the application of a number of mathematical methods from the sphere of correlation and frequency analysis which are well known from physiological and technical applications (Milsum, 1966; Leonov et al., 1965).

Material and Methods

In seven healthy women aged 31.2 ± 7.3 years with a body weight within the range of ± 10% of the ideal weight according to Broca three trials were made:

1. Trial O. During bed rest, blood specimens were collected from a cannula inserted into the cubital vein at 15-s intervals for a period of 6 min, a total of 24 samples, and serum GL and FFA were assessed.

2. Trial G. 50 g glucose were administered by mouth in 100 ml tea. After 30 min blood samples were collected from the cubital vein as in trial O.

3. Trial E. The experimental subjects were subjected to a load on a bicycle ergometer, the load being increased steadily to the point of exhaustion. The load started at 8.2 W and increased every 15 s by 8.2 W. The load ended between the 4th and the 6th min at the level of 131.2–196.8 W. During the 5th min of recovery blood samples were collected from the cubital vein with the subject in a recumbent position as in trial O.

All trials took place in the morning on fasting after an interval of one week, and their order was alternated individually at random.

In three healthy men aged 21.3 ± 0.4 years with a body weight of 90–100% ideal weight according to Broca two trials were made:

4. Trial F. Every subject was examined 6 times, always three times (at 10 a.m., 6 p.m., and 2 a.m.) on the control day and three times at the same times on the fourth day of complete fasting. On the control day a mixed diet was served providing 12,600 kJ, not later than 4 h before the time of blood collection. During each of the six examinations blood samples were collected from the cannula in the cubital vein and GL was assessed as in trial O. All 24 collections were, however, made concurrently from both cubital veins.

5. Trial T. Each subject was examined six times in the morning on fasting. During each of the six examinations from the cubital vein 24 consecutive collections were made and GL was assessed. In two trials the intervals between the collection of samples were 15 s, during the subsequent two 30 s and during the remaining two 60 s. The order of these trials between which there was an interval of at least three days was alternated at random.

GL was assessed by the ortho-toluidine method, and FFA according to Dole (1956). In trials O, G, and E the two parameters were assessed in triplicate. All calculations were made on Tesla 200 and Wang 2,200 computers.

Mathematical Methods

The starting point for mathematical computing was a 24-point series, $u_1 \ldots, u_n$ where each point (u_i) was the arithmetical mean of three assessed values.

In each series, the *arithmetic mean* of all 24 values ($\bar{u}$) was calculated as well as their *coefficient of variance* (cv%). The *variation range* in per cent ($VR\%$) was calculated from the equation

$$VR\% = \frac{u_{max} - u_{min}}{\bar{u}} \cdot 100 , \tag{1}$$

where u_{max} is the maximum and u_{min} the minimum value of the time series. The coefficient of variance in per cent (cvt%) for each triplicate of estimations was also calculated.

Dispersal analysis was used to see whether the scatter of values of the time series was significantly higher than the scatter in triplicates.

From each time series a *curve of sliding sums* was obtained, each having six points, and then by the method of minimum squares a regression line was drawn and the statistical significance of the possible trend was tested.

For each series an *autocorrelation function* (C_k) was found from the relationship

$$C_k = \frac{1}{n} \sum_{i=1}^{n-k} (u_i - \bar{u}) \cdot (u_{i+k} - \bar{u}) . \tag{2}$$

C_k is a function of k which acquires values of 0, 1, 2 . . ., $n - l$, whereby in practice we evaluate at the most $k = (n - 1)/2$ (Leonov et al., 1965).

In every series $u_1 \ldots, u_n$ the *periodogram I* (ω) was also calculated (Anděl, 1976) according to the equation

$$I(\omega) = \frac{1}{2\pi}(C_0 + 2 \sum_{k=1}^{n-1} C_k \cos k\omega) , \tag{3}$$

where C_k are values of the estimated autocorrelation function calculated from equation (2). The periodogram has high values in the points which correspond to angular velocities $\omega_1 \ldots, \omega_p$ of latent periodicities. The statistical significance of the highest values of the periodogram were tested by Fisher's test (Fisher, 1929).

From the calculated angular velocities $\omega_1 \ldots, \omega_p$ we obtain the time course of the periodic component, i.e. the regression function from equation

$$x_i = \sum_{k=1}^{p} (a_k \cos i\omega_k + b_k \sin i\omega_k) , \tag{4}$$

where coefficients a_k and b_k are estimated by the method of minimum squares which leads to formulae

$$a_k = \frac{2}{n} \sum_{i=1}^{n} u_i \cos i\omega_k , \quad b_k = \frac{2}{n} \sum_{i=1}^{n} u_i \sin i\omega_k . \tag{5}$$

For two parallel pairs of series $u_1 \ldots, u_n$ and $v_1 \ldots, v_n$ the *cross-correlation function* (CC_k) was calculated from the equation

$$CC_k = \frac{1}{n} \sum_{i=1}^{n-k} (u_i - \bar{u}) \cdot (v_{i+k} - \bar{v}) . \tag{6}$$

CC_k is (similarly as C_k) a function of k which may acquire also negative values. For $k > 0$ u lags after v (function "LAG"), for $k < 0$ u is ahead of v (function "LEAD"). The *standardized cross-correlation function* (NCC_k) is calculated from the equation

$$NCC_k = \frac{CC_k}{s_u \cdot s_v} , \tag{7}$$

where s_u and s_v are standard deviations of the two basic series.

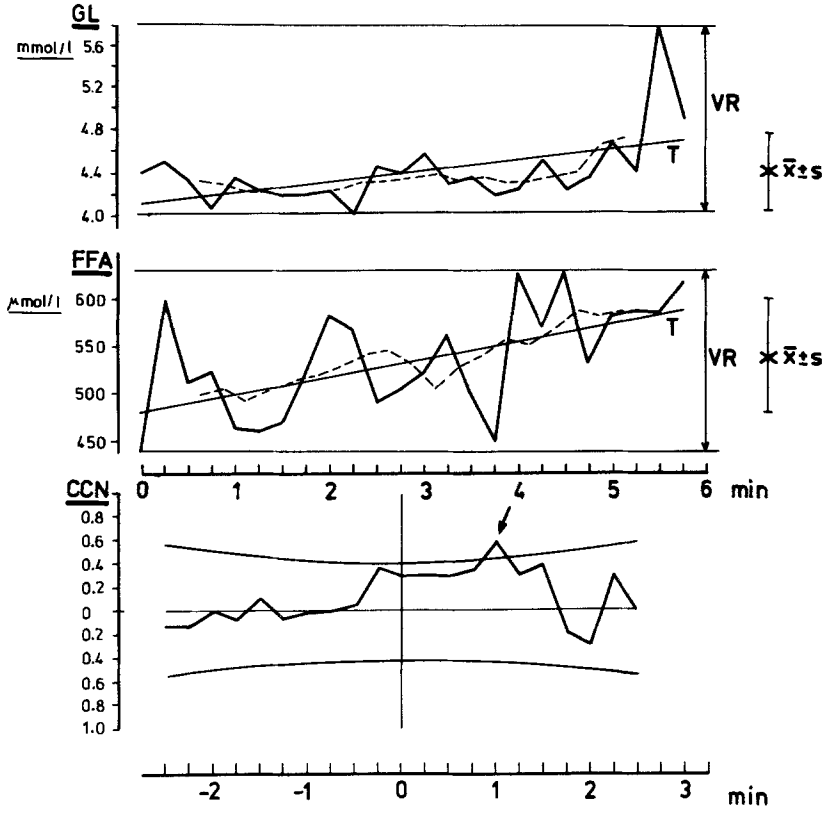

Fig. 1. Pairs of time series of the blood sugar level (GL) and free fatty acid level (FFA) and their mutual standardized correlation function (CCN). T = trend line (in GL and FFA statistically significant trend). Dashed line—curve of sliding sums of 6 values. VR = variation range, $\bar{x} \pm s$ mean value and its $\pm$ 1 standard deviation. In the correlation function the 95% zone of significance of values is indicated, significant correlations are indicated by an arrow

Results

The mean values $\bar{u}$ in mmol/l (mean coefficients of variance in per cent cv%/mean coefficients of variance in triplicates in per cent cvt%) of series GL were in experiment O, 4.68 (6.32/0.79), in trial G, 6.60 (6.56/0.96) and in trial E, 4.65 (4.62/0.88), and of FFA series in experiment O, 0.67 (15.82/3.21), in trial G, 0.53 (38.15/4.14), and in experiment E, 0.48 (17.23/4.12).

Figure 1 illustrates two time series GL and FFA in trial O; sliding sums, trends, variation range (VR), and scatter of values are also plotted. In the figure there is, moreover the cross-correlation function of the two time series including the range of significance (95% level).

Figure 2 summarizes VR of series GL and FFA in trials O, G, and E in absolute figures and per cent of mean values. VR in mmol are greater in GL than FFA, when expressed in per cent the ratio is reversed. The variation of values increases somewhat in trial G and declines in trial E, although the differences are not statistically

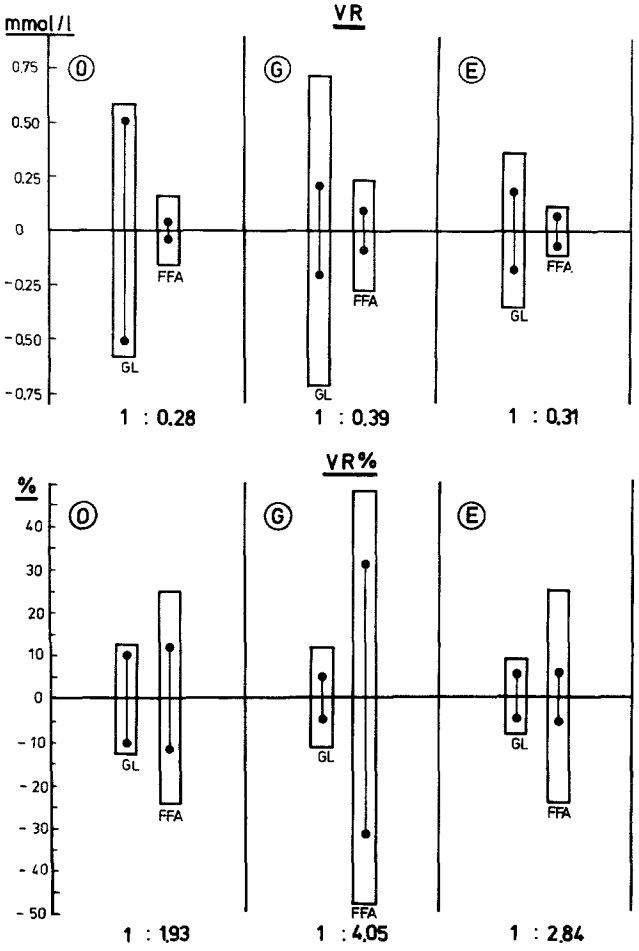

Fig. 2. Variation range of the blood sugar level (GL) and FFA in absolute values (VR) and in per cent (VR%) in trials on fasting (O), after glucose by mouth (G) and after exercise (E). The line inside the columns stands for 1 s

significant. cvt% of the triplicates is much smaller as compared to cv% of the time series. Dispersal analysis revealed that in trials O, G, and E the scatter of values of the time series is highly significantly greater ($p < 0.01$) than the scatter between the triplicates.

In trial O on fasting a trend was found in GL (rising or declining) which was significant at least at the 5% level in 37.5%, in FFA in 62.5%. In trial G after oral glucose the trend was significant in GL and FFA in 71.4%. In trial E after exercise a significant trend was found in GL in 71.4% and in FFA in 85.7%. The mean range of variation in per cent, VR%, declined markedly in the curves of sliding sums. The sliding sum of six values is equivalent to the prolongation of the period of blood collection from 15 s to 90 s. VR% declined by calculation of the sliding sums (by the sixfold protraction of the period of blood collection) in trial O for GL to 40.3 $\pm$

Table 1. Cross-correlation between glucose and free fatty acid blood levels

Cross correlation between glycemia and FFA	Experiment	"LAG"	"O"	"LEAD"	Sum
Positive correlation $p < 0.05$	O	3 (14.3%)	0 (0%)	1 4.8%)	4 (19%)
	G	1 (4.8%)	0 (0%)	1 (4.8%)	2 (9.5%)
	E	1 (4.8%)	2 (9.5%)	0 (0%)	3 (14.3%)
	$\sum$	5 (23.8%)	2 (9.5%)	2 (9.5%)	9 (42.8%)
Negative correlation $p < 0.05$	O	2 (9.5%)	0 (0%)	1 4.8%)	3 (14.3%)
	G	0 (0%)	4 (19.0%)	1 (4.8%)	5 (23.8%)
	E	1 (4.8%)	1 (4.8%)	1 (4.8%)	3 (14.3%)
	$\sum$	3 (14.3%)	5 (23.8%)	3 (14.3%)	11 (52.4%)
No correlation	O	–	–	–	–
	G	–	–	–	1 (4.8%)
	E	–	–	–	–
Sum		8 (38.1%)	7 (33.3%)	5 (23.8%)	21 (100%)

18.8% of the original value, in FFA to 50.5 ± 19.8%. In trial G VR% declined for GL to 58.3 ± 20.7%, for FFA to 72.7 ± 54.4%, and in trial E for GL to 41.9 ± 15.6% and for FFA to 60.2 ± 16.3%. The differences between trials are not significant.

The results of calculation of the cross-correlation function are given in Table 1 from which it ensues that mutual significant correlations exist in almost all instances, they are, however, positive as well as negative, more frequently with a shift than without.

Figure 3 is an example of the time series GL in trial O where, from the calculation of the periodogram, four harmonic components were obtained, the first of which is statistically significant. The figure records the original functions, 1, 1 + 2, 1 + 2 + 3, and 1 + 2 + 3 + 4 the harmonic component. The sum of four harmonic components is at the same time a regression function which is compared with the original course. Finally, also the residual components are plotted which again have a periodic character. Table 2 records the results of the analysis of GL and FFA by means of a periodogram in trials O, G, and E as revealed periods including their statistical significance.

Figure 4 illustrates two pairs of GL obtained by concomitant collection of blood from the left and right cubital vein. The first pair correlates significantly positively, the second one inversely and the third one inversely only after a mutual shift by 10 steps (150 s). From the total of 18 trials, complete results (all 48 values) were obtained in 11 instances, i.e., in 6 pairs from the control period and in 5 pairs from the period of fasting. Comparison of the mean values, scatter and variation coefficients (absolute values and percentage values) of these 11 pairs is summarized in Table 3. The mean values of GL from the left and right arm correlate positively ($r_{xy} = 0.991$;

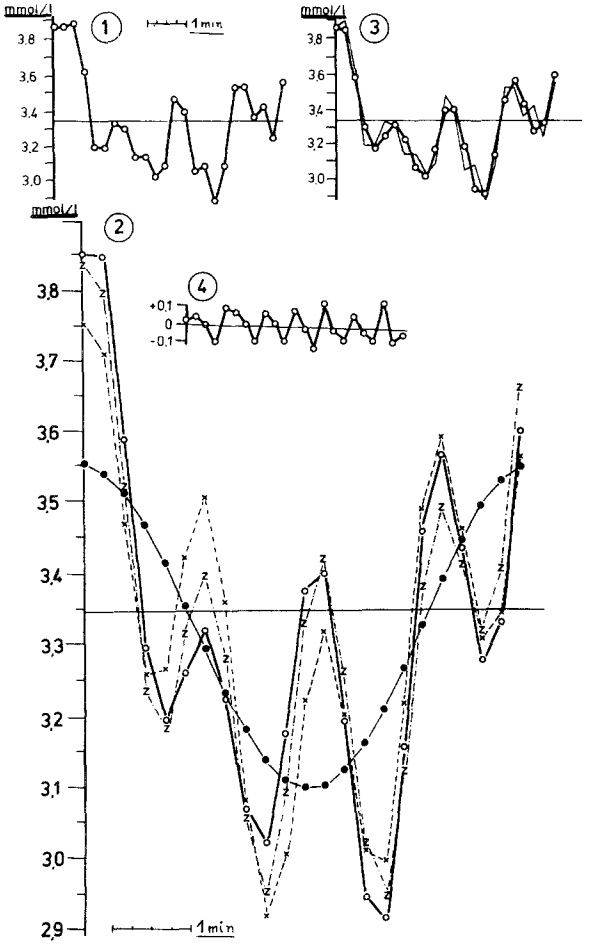

Fig. 3. Example of series GL from trial O (1). Part (2) records four harmonic components calculated by means of a periodogram (1 black circles, 1 + 2 ×, 1 + 2 + 3 z and 1 + 2 + 3 + 4 white circles). Part (3) compares the regression function which was obtained by adding four harmonic components with the original time series. Part (4) shows the residual deviation, i. e., the difference of the original series and regression function

$p < 0.001$), as do the scatter ($r_{xy} = 0.913$; $p < 0.001$) and VR ($r_{xy} = 0.767$; $p < 0.01$).

During the period of fasting the mean value of GL is markedly lower and the variation range in mmol also declines proportionately (the mean GL correlates positively with VR $r_{xy} = 0.9753$; $p < 0.01$), while VR% practically does not differ. From the results we cannot draw a reliable conclusion as to whether the parameters differ in the course of 24 h, i.e., whether they have a circadian rhythm. Correlation analysis revealed that the series of GL from the left and right arm are not correlated on four occasions, that they correlated positively four times (including twice with a shift), and that they correlate inversely three times (including twice with a shift).

Table 2. Results of the analysis of glucose and free fatty acid series by means of a periodogram. + = statistical significance of detected period on 5% (or higher) level of significance

Experiment	Glucose and FFA series	Period (s)							
		Glucose				FFA			
O	1	172.5				86.2			
(on fasting)	2	86.2				69.0			
	3	34.5				345.0			
	4	172.5				345.0+	172.5+	69.0	
	5	345.0				86.2+	49.4		
	6	172.5				345.0			
	7	345.0+	115.0			345.0			
G	8	345.0				172.5			
(after glucose	9	345.0+	172.5	115.0	43.2	345.0+	172.5+	31.4	
per os)	10	72.0				172.5+	69.0		
	11	86.2+	345.0	172.5	115.0	86.2			
	12	115.0				57.4			
	13	172.5				345.0	172.5		
	14	86.2	172.5			172.5			
E	15	49.4				345.0+	115.0		
(recovery after	16	345.0+	115.0			69.0			
exercise)	17	345.0	115.0			345.0+	69.0		
	18	115.0				345.0			
	19	115.0				172.5			
	20	86.2+	172.5	43.2		345.0+	172.5	34.5	
	21	86.2				345.0+	34.5		
Significant periodicity in % of total		23.81%				38.10%			

Figure 5 shows the results of trial T, where gradually the intervals between the 24 collections for GL are increased. The variation range in mmol and the per cent increases with increasing intervals are shown but the differences are not significant.

Discussion

Oscillations of various physiological parameters have been observed and studied in detail for some time. One example is the reported variations of blood pressure and heart rate under conditions of complete rest (Symposium, 1977). Variations of a similar type were described also in repeated estimations of blood sugar level (Hansen, 1923; Iberall et al., 1968) of free fatty acids (Court et al., 1971), and of serum insulin (Bessman et al., 1973) etc. after intervals several seconds. Oscillations were found also in tissue and cell enzyme activities (Chance and Schooner, 1964; Ghosh and Chance, 1964; Goldbetter and Lefever, 1972 and others).

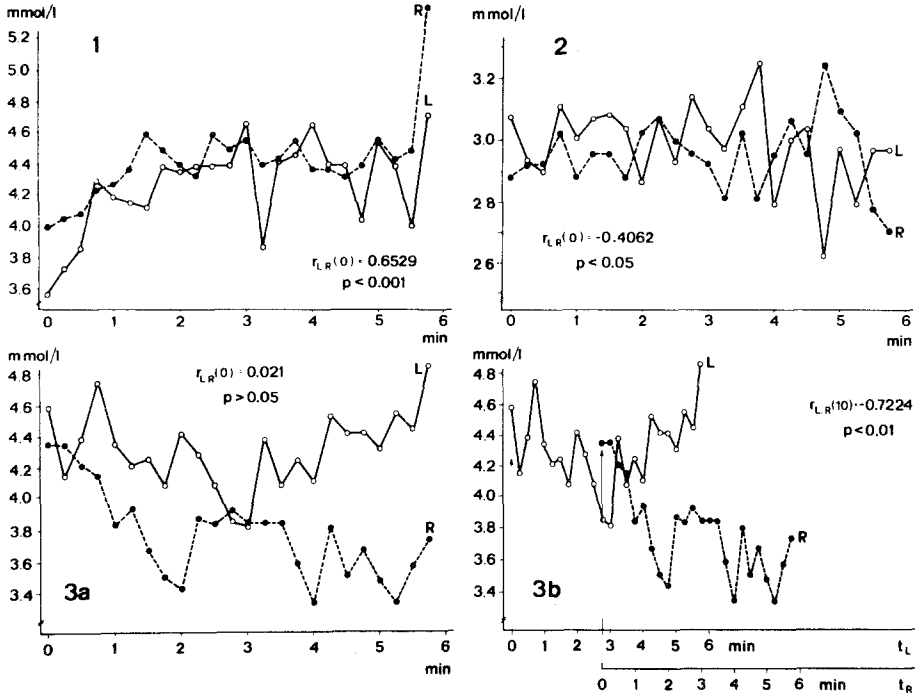

Fig. 4. Example of three pairs of 24-point blood sugar curves (in mmol/l) collected parallel after 15 s from the left (L) and right (R) upper extremity in trial F. 1 = Standardized correlation function is without shift (0) highly significantly positive. 2 = Standardized correlation function is without shift (0) significantly negative. 3 = Standardized correlation function without shift (0) is not statistically significant. 3a = Standardized correlation function of the same pair with shift by 10 steps/150 s is highly significantly negative

GL and FFA blood levels are the result of a dynamic equilibrium which is under very sensitive control (Eisenstein and Singh, 1973). The feedback principle is involved (Milsum, 1966; Oppelt, 1964), a typical property of which is "regulation by error".

The blood sugar level is regulated both as regards supply and removal. On the one hand there is insulin (Eisenstein and Singh, 1973) which reduces glucose release from the liver and enhances its uptake in the periphery and leads to a decline of the blood sugar level. A raised blood sugar level causes a parallel increase of insulin production and a reduction of glucagon formation. On the other hand, there is glucagon (Lefevbre and Unger, 1970) which enhances gluconeogenesis and glycogenolysis, reduces the glucose uptake in the periphery and increases the blood sugar level. The reduced blood sugar level causes a parallel rise of glucagon production and reduces insulin formation. A number of other factors act synergistically with glucagon, such as the orthosympaticus, catecholamines, in some respects also corticoids, STH, thyroxine (Eisenstein and Singh, 1973).

The mobilization of FFA is influenced in particular by adrenalin and other factors, such as glucagon, STH, thyroxin, FMS (Eisenstein and Singh, 1973). Adrenalin activates adenylate cyclase in the membrane of fat cells and this leads to

Table 3. Results of the trial F. Mean values ($\bar{u}$), scatter (s^2), absolute and relative variation ratios (VR, $VR\%$) in blood glucose series (GL) obtained concomitantly from the left (L) and right (R) cubital at different hours of the day (H)

Period	GL series (h)	$\bar{u}$ (mmol/l)		s^2 (mmol/l)^2	VR (mmol/l)	$VR\%$ (%)
Control	1 (10)	L	4.26	0.020	0.61	14.2
		R	4.29	0.061	1.32	30.9
	2 (18)	L	4.30	0.062	1.07	24.8
		R	3.70	0.085	1.01	27.7
	3 (02)	L	4.25	0.030	0.76	18.1
		R	4.28	0.063	1.31	30.9
	4 (18)	L	7.04	0.175	1.85	22.0
		R	7.01	0.223	2.06	29.4
	5 (10)	L	4.26	0.090	1.18	27.7
		R	4.41	0.070	1.45	32.7
	6 (10)	L	5.65	0.065	1.27	22.4
		R	5.58	0.030	0.87	15.5
Fasting	7 (10)	L	3.02	0.026	0.70	23.0
		R	3.01	0.014	0.39	13.1
	8 (10)	L	2.35	0.011	0.38	16.1
		R	2.36	0.011	0.45	19.1
	9 (18)	L	3.08	0.017	0.58	19.0
		R	3.28	0.027	0.80	24.3
	10 (02)	L	2.41	0.017	0.47	19.4
		R	2.38	0.013	0.57	23.8
	11 (02)	L	2.99	0.016	0.63	21.0
		R	2.96	0.013	0.53	18.2

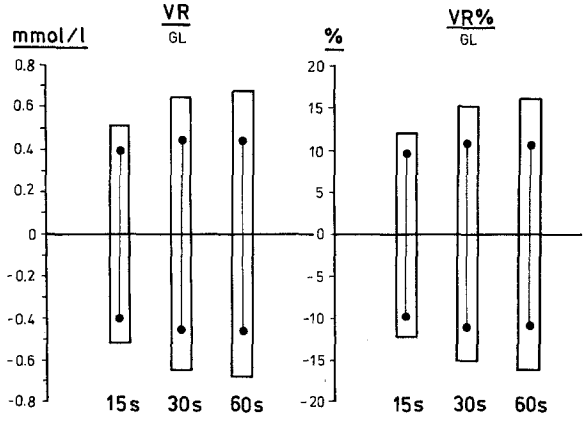

Fig. 5. Absolute and relative variation range of the blood sugar level (GL) VR and VR% during 24 blood collections with intervals of 15, 30, and 60 s in trial T

an increased formation of cyclic AMP. cAMP activates the breakdown of triglycerides in the cell to glycerol and fatty acids. At the same time the release of arachidonic acid is activated which increases the formation of prostaglandin E_2 (PGE_2). The latter then inhibits adenylate cyclase and may activate phosphodiesterase which enhances the breakdown of cAMP. Both these mechanisms, mediated by PGE_2, thus inhibit lipolysis (Horton, 1969; Editorial, 1977).

The glucose and FFA levels are thus regulated by double feedback systems. The rise in level of the regulating substance is as a rule associated with a drop of activity of the counterregulating substance and vice versa. The regulating process can therefore be simulated, e.g., by mutually influencing oscillations of two counteracting leading factors which are mutually relatively closely linked with a certain phase shift.

The position is, moreover, complicated by the fact that glucose and fatty acids also influence each other (Randle's cycle – Randle et al., 1963) and thus the relationship of changes of their levels is frequently inverse. The harmony of all the above factors is so complicated that oscillations need not be very regular and their periodic nature may be masked by some "interfering" processes. The results indicate that the periodogram (Anděl, 1976) is for short time series probably the most effective method with the greatest detection rate of periodic components of investigated processes. The use of periodograms made it possible to detect a statistically significant periodic component in one quarter of the investigated blood sugar and in one third of the FFA level series.

So far we anticipated that the cause of oscillations are vibrations of feedbacks in the investigated systems. This would influence the supply and removal of substances to and from the distribution volume but not "mixing" of substances in the blood stream and body fluids. Proper mixing of substances in the distribution volume would mean that in the entire circulation there would be a uniform "level" of the substance and during parallel detection of the concentration at various sites of the circulation the same concentrations would be recorded which could oscillate synchronously or with a constant time shift. Our results with parallel collections of blood samples from both cubital veins do not suggest this, as in one third of the cases the curves did not correlate at all and the remaining two-thirds contain a mixture of positive and negative correlations which are difficult to sort out.

These findings may suggest that in some instances glucose (and FFA, resp.) is not released continuously into the blood stream, but in "quanta". Then the "blood level of substances" would be rather an unevenly mixed sequence of "boli" of different concentrations. The finding of a certain concentration of a substance at a given site in the circulation at a given moment then would not be a deterministic but a stochastic phenomenon. If this assumption is correct, then the oscillation of the concentration should be the smaller, the better the substance is mixed, i.e., the smaller its turnover. This would be consistent with the greater range of variation in per cent in FFA with a more rapid turnover as compared with the blood sugar level. Thus arising oscillations could have a quite casual character and the periodic component of the oscillations would be lacking.

Hitherto, assembled results suggest that in the development of oscillations both factors (i.e., inadequate mixing and vibrations caused by the feedback) are involved simultaneously. It can not be ruled out that other causes of oscillations may exist. In

the blood sugar level and FFA level we certainly cannot neglect the finding of regularly encountered important correlations between the blood sugar level and FFA which are known to influence each other in an important way (Randle et al., 1963). The actual effect of these relations is so far obscure, as the cross-correlations of the two parameters can positive or negative with equal probability.

The size of oscillations is, no doubt, influenced also by the site of collection of the blood sample. However, with the exception of a single finding of parallel oscillations of arterial and venous blood (Iberall et al., 1968) data on this factor are lacking. A surprisingly small effect on oscillations was produced by such intense factors interfering with the steady state, e.g., fasting, oral doses of glucose, or exercise.

The magnitude of variations recorded during the 6-min investigation apparently is not complete in some cases. This is suggested by the existence of significant trends. The latter are no surprise after exercise and in particular after administration of glucose, when the mean levels return gradually to equilibrium at rest. Significant trends were, However, recorded also during absolute rest. Perhaps the rhythms interfere with a period longer than 6 min. This is suggested also by the marked (although insignificant) increase in the range of variation when the intervals between blood collections and thus the total observation period in trial T are prolonged. In addition to short-term variations, 24-h rhythms exist in blood sugar level, FFA, and insulin (Sollberger, 1965) which, however, probably play no part during the brief period of investigation. Similarly, the magnitude of oscillations was not influenced by the time of collection of the sample in trial F.

The questions submitted in the introduction can thus be answered for the time being as follows:

a) Oscillations of GL and FFA are present in all instances, they are of a higher order than the error of the method and thus are not merely an incidental finding.

b) In the majority of cases it is possible to prove in the oscillations a periodic component which was, however, statistically significant only in some cases. The length of the period detected during the 6-min investigation differs, however, and periods of 2, 3, and 6 min predominate. The existence of longer periods hight be revealed by longer investigations.

c) Oscillations of GL and FFA probably influence each other, but the nature of this relationship is at present obscure.

d) Hitherto, assembled results suggest that inadequate mixing as well as vibrations caused by the existence of negative feedbacks participate in the development of oscillations. The effect of other factors cannot be ruled out.

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