tinal cancer (*10*). Some patients have cellular immunity to the skin reactive antigen (*1, 2*). It remains to be determined how these reactions correlate with each other and with the clinical state of the patients. Purified CEA preparations have failed to produce blast transformation of the lymphocytes of patients with intestinal cancer (*5*). These CEA preparations have also been inactive in migration inhibition assays (*11*). It will be of interest to test the skin reactive antigen in these assays. Using the colony inhibition assay, Hellstrom *et al.* (*12*) have found reactivity, in the lymphocytes of patients with intestinal cancer, against antigens common to human colonic carcinomas and fetal gut. Whether the antigen detected by this assay is the same as either CEA or the skin reactive antigen, or whether there is a third type of carcinoembryonic antigen in human intestinal cancers remains to be determined.

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References and Notes

1. A. Hollinshead, D. Glew, B. Bunnag, P. Gold, R. Herberman, *Lancet* **1970-I**, 1191 (1970).
2. R. B. Herberman, A. Hollinshead, T. C. Alford, *Proceedings of the First Conference and Workshop on Embryonic and Fetal Antigens in Cancer*, N. G. Anderson and J. H. Coggin, Jr., Eds. (Oak Ridge National Laboratory, Oak Ridge, Tenn., 1971), p. 331.
3. P. Gold and S. Freedman, *J. Exp. Med.* **122**, 467 (1965).
4. P. Gold, M. Gold, S. Freedman, *Cancer Res.* **28**, 1331 (1968).
5. M. C. Lejtenyi, S. O. Freedman, P. Gold, *Cancer* **28**, 115 (1971).
6. J. Krupey, T. Wilson, S. O. Freedman, P. Gold, *Immunochemistry* **9**, 617 (1972).
7. G. L. Wright, K. B. Farrell, D. B. Roberts, *Clin. Chim. Acta* **32**, 285 (1971).
8. C. G. McWright, thesis, George Washington University (1970).
9. D. Thomson, J. Krupey, S. Freedman, P. Gold, *Proc. Nat. Acad. Sci. U.S.A.* **64**, 161 (1969).
10. P. Gold, *Cancer* **20**, 1663 (1967).
11. W. H. Churchill, unpublished observation.
12. I. Hellström, K. E. Hellström, T. H. Shepard, *Int. J. Cancer* **6**, 346 (1970).
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Cyclic Changes in Insulin Needs of an Unstable Diabetic

Abstract. *The fluctuating insulin requirements of an unstable diabetic over an 8-year period have been subjected to spectral analysis. There is evidence of cyclic changes of several different period lengths in addition to red noise. The periodicities indicate that social causes play no major role but suggest that a weather-mediated effect may exist.*

Diurnal variations in glucose tolerance have been established (*1*) and annual variations in the incidence and severity of diabetes have long been known (*2*). In addition there is a large variance in the glucose tolerance of unselected individuals from day to day (*3*), and one type of diabetic, the unstable (brittle or labile) diabetic, displays wide variations on the scale of hours to days (*4*) in the amount of insulin required to maintain normoglycemia. It is our intention to show that these variations, for one unstable diabetic, are not entirely random but exhibit deterministic changes of several different cycle periods, and a strong red-noise or $1/f$ trend (*5*) in their spectrum (f = frequency).

One of us (M.J.C., age 34) is an unstable diabetic who has kept records of the quantity of insulin, mostly NPH or Ultralente, he requires each day. The dosage has been adjusted, incrementally several times a day as required, to maintain normal blood sugar levels while the dietary intake of carbohydrate was kept constant by estimation and taken in regularly divided portions. The criterion of "normal" blood sugar was originally the absence of subjective hypoglycemia (roughly, pro-

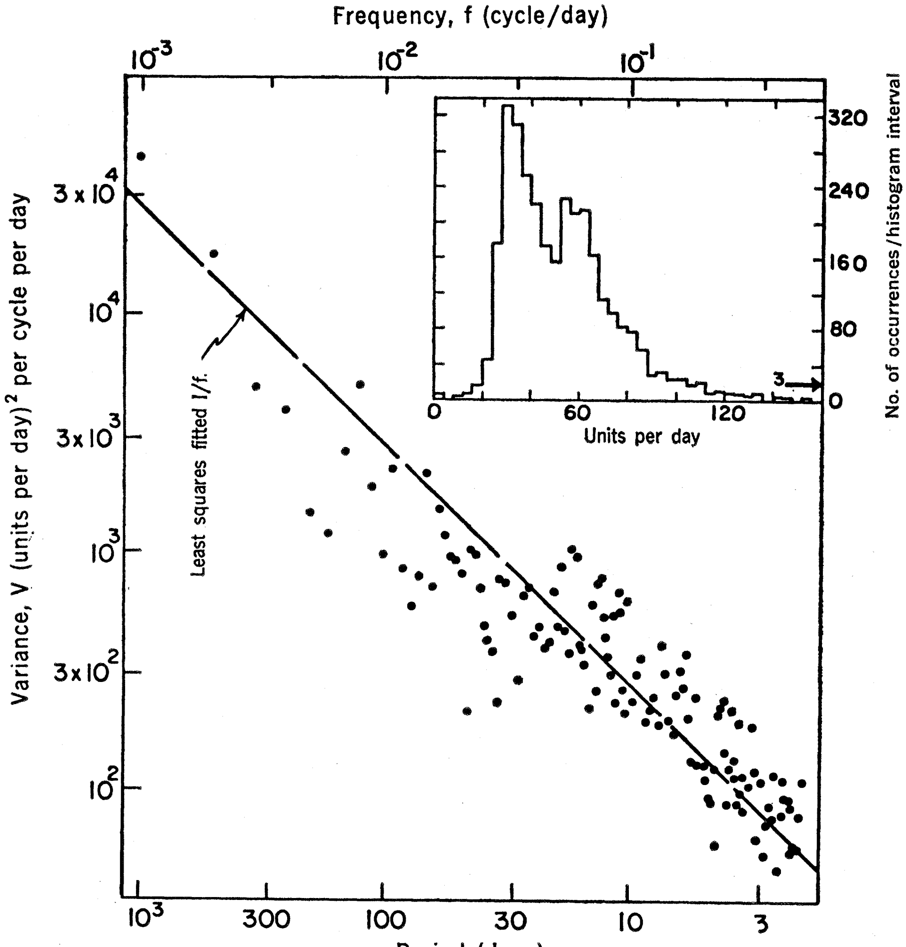

Fig. 1. The variance spectrum of the time series formed by 3072 consecutive daily insulin doses. In this log-log plot of the variance density against frequency, the 512 points of the raw spectrum have been grouped for clarity. The first 30 points are untreated; points 31 to 100 are averaged in pairs; points 101 to 300 are averaged in fives; points 301 to 512 are averaged in tens. (Inset) The distribution of 3109 daily doses of insulin, in insulin units per day. There are three doses in excess of 160 units per day.

longed blood sugar levels below 50 mg/100 ml) together with the absence or trace excretion of urinary glucose, assessed several times daily with semiquantitative oxidase tape (Testape, Eli Lilly & Co.). Since 1965 these have been supplemented by oxidase capillary blood sugar tests (Dextrostix, Ames Co.) performed several times daily. The distribution of consecutive daily doses from 22 November 1962 is shown in Fig. 1 (inset). Defining an "ideal dose" as one which would preserve normoglycemia when the dietary intake was unchanging from day to day, we believe that these daily doses mostly depart less than 10 percent from the ideal dose.

We have calculated the variance spectrum (*6*) of 3072 sequential daily doses from 22 November 1962 to 20 April 1971, using linear interpolation to replace missing data for the month of September 1965. To facilitate use of the fast Fourier transform (*6*), the data were analyzed in three contiguous sets of 1024 days and the resulting spectra were added to obtain the raw variance spectrum shown in Fig. 1 as a log-log plot against frequency (*f*, in cycles per day). There is a clear trend for the variance to be inversely proportional to the frequency, constituting a so-called $1/f$ noise which is an example of red noise. Noise of this type has been noted in many other contexts ranging from rainfall (*5*) to the membrane potentials in resting nerve fibers (*7*).

To emphasize the spectral peaks we have subtracted the trend line shown in Fig. 1. Since the peaks are not intrinsically narrow, we have reduced the scatter by taking a five-point moving average; the resulting spectrum, with the lowest frequencies excluded, is shown in Fig. 2. The standard deviation of a point of Fig. 2 is about 31 (units per day)^2 cycle^{-1} day^{-1}. The spectrum at the lowest frequencies cannot be conveniently plotted on the same scale as Fig. 2, but displays two peaks, at 32.5 days and 26.3 days, comparable to that at 16.9 days in Fig. 2.

Table 1. The ten strongest peaks cataloged according to harmonic families to which they could belong. The uncertainty in all frequencies is ± 0.0025 cycle/day.

Harmonic family	Harmonic number	Frequency (cycle/day)	Period (days)
A	1	0.0308	32.5 ± 2.5
	2	.0591	16.9 ± 0.7
	3	.0907	11.02 ± 0.30
B	1	.0380	26.3 ± 1.6
	2	.0758	13.20 ± 0.40
	5	.1925	5.19 ± 0.07
C	4	.1366	7.32 ± 0.13
	5	.1689	5.92 ± 0.08
	7	.2355	4.25 ± 0.05
D		.1608	6.22 ± 0.10

Table 1 lists the ten strongest peaks in the whole spectrum. Nine of the peaks may be construed as belonging to harmonic families as shown, although such an association cannot be proved. The prominent peak at the 7.32-day period (0.1366 cycle/day) is significantly displaced from the 7.00-day period which would be anticipated as a consequence of many social mechanisms; the spectrum shows no peak near 7.00 days (0.1429 cycle/day). The 7.32-day period is close to one-half the 14.765-day (half the lunar synodic month) periodicity which appears prominently in rainfall records (*8*) and in the biological processes of certain plants and animals (*9*). A 17-day period in beard growth has been reported (*10*).

The probability of the partial spectrum shown in Fig. 2 arising by chance from a process which is in fact merely random can be estimated. The spectrum of a process in which each day's dose is unrelated to the dose needed on any other day is known (*6*) to have its variance density distributed approximately (*11*) as χ^2_{10} when smoothed by five-point averaging. Such spectra generally appear jagged but will exhibit no trends. If three such spectra are summed, in the way used to generate Fig. 2, the distribution will be approximately χ^2_{30}, and we confirmed this result for the marginal distribution of Fig. 1 (inset) by randomly shuffling the original data points.

The χ^2_{30} distribution will also approximately hold true for a spectrum derived by subtracting the $1/f$-noise trend from an original spectrum that consisted of the sum of a $1/f$ noise and a white noise. However, in the observed spectrum of Fig. 2 the variance density histogram does not conform to the shape of a χ^2_{30} distribution or any other χ^2 distribution and the best fit to χ^2_{30} gave rise to $\chi^2_6 = 101$, $P \ll .01$. We conclude that Fig. 2 does not represent the spectrum of a random process because of the existence of peaks, notwithstanding that some of

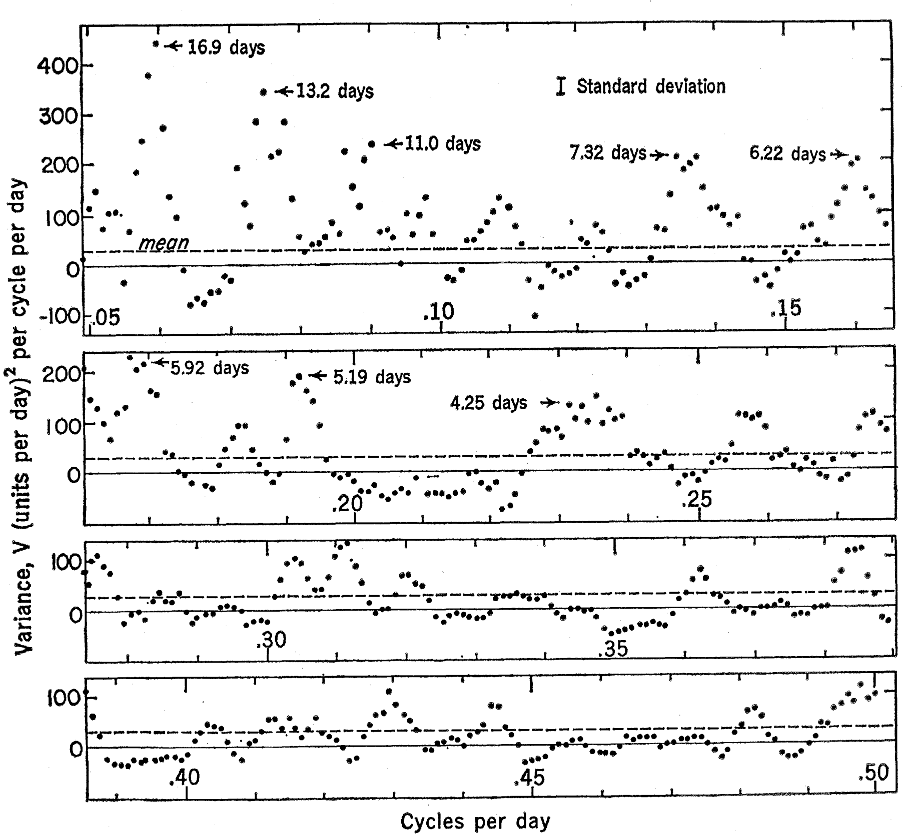

Fig. 2. Residual variance spectrum at frequencies greater than 0.05 cycle/day after subtraction of the $1/f$ trend and smoothing by a five-point moving average. The dotted line is the mean of the points shown and the abscissa is the mean of the entire residual spectrum before truncation at 0.05 cycle/day. Removal of trends is less complete at frequencies below 0.05 cycle/day, although peaks are still apparent.

these peaks may have arisen by chance.

The contribution of the ten peaks of Table 1 to the total variance of the data is about 10^{-2}. A large portion of the variance is attributable to relatively slow changes, the low frequency components which make up the red-noise trend. The residuum after subtraction of the red-noise is a near-white spectrum of which the peaks contribute 6 percent. None of the peaks represent a constant, strictly periodic process; they vary in amplitude and for some time spans any given peak may be undetectable.

Although the peaks are statistically significant and must represent the effects of deterministic processes, either endogenous or exogenous, on the insulin requirements of this diabetic, this analysis cannot shed any real light on the nature of these processes. Periodicities of apparently endogenous origin in 17-ketosteroid excretion of a male at 6.9 to 7.0, 17 to 21, and 29 to 31 days have been observed by Halberg *et al.* (*12*). However, the notable absence of a peak at a 7-day period provides strong evidence against a role for social mechanisms. The $1/f$ trend and the lunar-related peaks both raise the possibility that weather-related phenomena are responsible.

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References and Notes

1. A. J. Bowen and R. L. Reeves, *Arch. Intern. Med.* **119**, 261 (1967); R. Ben-Dyke, *Diabetologia* **7**, 156 (1971).
2. S. W. Tromp, *Medical Biometeorology* (Elsevier, Amsterdam, 1963), p. 560.
3. G. W. McDonald, G. F. Fisher, C. Burnham, *Diabetes* **14**, 473 (1965).
4. J. Pirart, *Diabetologia* **7**, 283 (1971).
5. B. B. Mandelbrot and J. R. Wallis, *Water Resour. Res.* **4**, 909 (1968).
6. G. M. Jenkins and D. G. Watts, *Spectral Analysis and Its Applications* (Holden-Day, San Francisco, 1968).
7. A. A. Verveen and H. E. Derksen, *Proc. Inst. Elec. Electron. Engr.* **56**, 906 (1968).
8. G. W. Brier and D. A. Bradley, *J. Atmos. Sci.* **21**, 386 (1964); E. E. Adderley and E. G. Bowen, *Science* **137**, 749 (1962).
9. F. A. Brown, Jr., *Proc. Soc. Exp. Biol. Med.* **120**, 792 (1965); ———, Y. H. Park, J. R. Zeno, *Nature* **211**, 830 (1966).
10. R. S. Sothern, *Proceedings of the International Society of Chronobiology* (meeting held in Little Rock, Arkansas, 1971), in press.
11. This result is strictly true if the marginal distribution is normal; the random process then constitutes "white noise." However, the result is not very sensitive to the form of the marginal distribution.
12. F. Halberg, M. Engeli, C. Hamburger, D. Hillman, *Acta Endocrinol.* **103** (Suppl.) (1965).
13. We thank Martin O. Harwit and André T. Jagendorf for their comments on the manuscript.

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Calcium Oxalate Crystals in the Aragonite-Producing Green Alga Penicillus and Related Genera

Abstract. *Calcium oxalate crystals occur in the marine green algae* Penicillus, Rhipocephalus, *and* Udotea, *known as producers of sedimentary aragonite needles. In contrast to the externally deposited aragonite crystals which are generally < 15 micrometers long, the oxalate crystals are larger (up to 150 micrometers) and are located in the vacuolar system of the plant. No calcium oxalate was found in the related but noncalcifying genera* Avrainvillea *and* Cladocephalus.

Species of the green algal genus *Penicillus* and of the related genera *Rhipocephalus* and *Udotea* (Codiales) are significant producers of fine aragonite needles in marine sediments (*1, 2*). *Penicillus* is a major contributor of aragonite lime mud in the Florida Bay and near shore parts of the Florida reef flat, while *Rhipocephalus* and *Udotea* are more abundant in the reef tract and the outer margin of Florida Bay (*2*). The algae deposit aragonite as an external lime sheath on the surface of the cell wall and, upon death of the organism, the sheath disintegrates into individual crystals. The aragonite crystals have been described as needles < 15 μm long (*2, 3*); small prisms approximately 0.5 μm long and serrated crystals about 1.0 μm long also occur (*4*).

We found that, in addition to the externally deposited aragonite, intracellular crystals are also present, often in significant quantities. These are calcium oxalate needles up to about 150 μm long and are produced in the vacuolar system of the organism.

In the light microscope the crystals appear as single acicular structures (Fig. 1, b to i). The internal structure of a plant with intracellular crystals is shown in a living, young uncalcified capitular filament of *Penicillus capitatus* (Fig. 1b). The cytoplasm, containing numerous green plastids, appears as a thin, continuous parietal layer. The crystals are randomly oriented in the central vacuolar system (*5*). In the transmission electron microscope (Fig. 1j) the crystals appear to be encased in a chamberlike structure within the granular vacuolar material. Their appearance is similar to the electron microscopic image of calcium oxalate crystals in higher plants (*6, 7*).

For x-ray diffraction analysis the intracellular crystals were isolated from specimens of *P. dumetosus*, collected in the Florida Bay, by dissolution of the aragonite sheath in warm 65 percent acetic acid and subsequent digestion of the organic matter in 5.25 percent commercial sodium hypochlorite solution at 70°C. The acetone-washed and dried sample was mounted in a Lindemann glass capillary and photographed with Ni-filtered CuKα radiation in a Debye-Scherrer powder camera of 57.3 mm radius (Fig. 1a).

Visually estimated approximate intensities and observed *d*-spacings for our sample are listed in Table 1. There

Table 1. Diffraction pattern of intracellular crystals in *Penicillus dumetosus* and of $CaC_2O_4 \cdot H_2O$ form B from ASTM powder diffraction file 14-770.

P. dumetosus		$CaC_2O_4 \cdot H_2O$	
d-Spacing (Å)	Intensity (I/I_0)	*d*-Spacing (Å)	Intensity (I/I_0)
8.6	10		
6.2	80	6.13	80
4.4	40	4.34	40
3.9	15	3.84	40
3.67	15		
3.35	5		
3.08	18	3.07	40
2.77	100	2.75	100
2.41	30	2.40	60
2.34	10		
2.24	40	2.22	60
2.20	3		
2.12	20	2.10	40
2.02	10	1.99	10
1.95	20	1.94	20
1.89	20	1.89	60
1.83	20	1.82	40
1.78	4		
1.74	20	1.73	40
1.69	10	1.69	10
1.65	1		
1.62	2		
1.58	3		
1.56	10	1.56	10
1.50	10		
1.48	5	1.48	40
1.43	3		
1.42	5	1.42	20
1.385	15	1.38	40
1.315	10	1.32	20
1.268	1		
1.235	1	1.23	10
1.190	10	1.19	40
1.170	4	1.17	10
1.150	10	1.15	40
1.130	2		
1.115	1		
1.092	5	1.09	10
1.060	10	1.06	20
1.035	2		
1.014	2		
0.988	2		
0.951	1		
0.930	3		
0.845*	3		
0.788	2		

* Diffuse