References and Notes

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9. For hybridization in situ to detect DNA, tissue sections cut with a cryostat from frozen tissue were fixed in a mixture of ethanol and acetic acid, and subsequently treated with 0.2*N* HCl. RNA was removed enzymatically with pancreatic ribonuclease [100 μg per microliter of sodium saline citrate, double strength (2 × SSC), 37°C, 30 minutes], and the DNA was denatured in 95 percent formamide 0.1 × SSC (65°C, 2 hours). The complementary DNA (cDNA) was hybridized under a cover slip at a concentration of 4 ng per 5 μl in 4 × SSC containing 50 percent formamide at 45°C to a C_0t of 2 mole/liter per second, based on the cDNA in solution. The slides were then washed in buffer (30 m*M* sodium acetate, *p*H 4.5, 100 m*M* NaCl, 3 m*M* $ZnCl_2$), treated with 10 units of S1 nuclease (Sigma) per milliliter for 2 hours at 50°C, washed, dried, and coated with Ilford K2 emulsion. After radioautographic exposure, the slides were developed and stained with Giemsa (*8*) (A. Haase and L. Stowring, in preparation).
10. A relatively symmetric transcript complementary to the RNA genome of visna was synthesized in an endogenous reaction containing actinomycin D. Reaction mixtures (1 ml) contained 50 μmole of tris-HCl, *p*H 7.4, 8 μmole of $MgCl_2$, 10 μmole of dithiothreitol, 0.1 mmole of dATP, dGTP, dCTP, and ^{3}H-labeled dTTP (specific activity 59.5 c/mmole, 10^8 dpm per microgram of DNA), 100 μg of actinomycin D, 250 μg of purified visna virus, and 0.02 percent Triton X-100. After incubation at 37°C for 4 hours, the reaction products were purified, treated with 0.6*M* NaOH for 4 hours at 37°C, neutralized and passed over Sephadex G-50 to remove free isotope and unlabeled nucleotides. The probe fractions in the exclusion volume were pooled and precipitated with ethanol. After recovery by centrifugation, the ^{3}H-labeled probe was suspended in a small volume of buffer (10 m*M* tris-HCl, *p*H 7.4, 1 m*M* EDTA) at a concentration of 2 ng/μl. At least 90 percent of the predominantly single-stranded DNA transcript contained sequences complementary to viral RNA, determined by annealing the DNA to a vast excess of viral RNA to a Cr_0t (concentration of ribonucleotide in moles per liter times the time in seconds) of 2; the DNA probe complementary to virion RNA was representative of the entire genome, as it protected 60 to 100 percent of labeled 70*S* RNA from digestion by ribonuclease at mass ratios of cDNA/RNA of less than 10 [A. Haase, A. Garapin, A. Faras, H. Varmus, J. Bishop, *Virology* **57**, 251 (1974)].
11. SCP cells synchronously infected in vitro contain, late in the viral growth cycle, an average of 100 to 200 copies of viral DNA per cell, measured by liquid annealing methods [(*2*) and B. Traynor, A. Haase, M. Brahic, in preparation]. Under the same conditions of infection the viral DNA content of SCP cells infected in vitro measured by in situ hybridization corresponds to 20 to 40 grains per nucleus (L. Stowring and A. Haase, unpublished data).
12. The deoxyribonuclease treatment was at 200 μg/ml in buffer containing 10 m*M* $MgCl_2$, 37°C, 1 hour.
13. In the competition hybridizations, SCP DNA from uninfected cells, or cells infected for 72 hours with about 150 copies of proviral DNA per cell, was extracted, sheared (*2*), and added to the hybridization buffer along with the 4 ng of [^{3}H]cDNA. To accommodate the 300 μg of unlabeled DNA, the volume was increased to 50 μl. See (*9*) for subsequent hybridization procedures.
14. The major polypeptide of visna virus, like other RNA type C viruses, has a molecular weight of about 30,000 and is designated p30 [J. August, D. Bolognesi, E. Fleissner, R. Gilden, R. Nowinski, *Virology* **60**, 595 (1974), for nomenclature]. The p30 was purified to homogeneity by isoelectric focusing; virus preparations were dissociated in Brij detergent and urea and focused successively in broad (*p*H 3 to 10) and narrow (*p*H 5 to 8) gradients. Material with an isoelectric point of 6.9 was freed of ampholines by desalting on Sephadex G-25, and concentrated by lyophilization. The final product was shown by electrophoresis in gels of polyacrylamide to consist of a single species corresponding in migration to p30. This material was used to immunize guinea pigs. The resultant antiserums reacted with visna virus and p30 in immunodiffusion, but not with other viral antigens, sheep cell extracts, or serum proteins. In indirect fluorescent antibody tests it stained SCP cells infected with visna virus in vitro, but not uninfected SCP cells or tissues; and this staining could be blocked specifically by prior adsorption of the serums with p30.
15. A. Haase and L. Stowring, in preparation.

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Insulin, Glucagon, and Glucose Exhibit Synchronous, Sustained Oscillations in Fasting Monkeys

Abstract. *In overnight fasted rhesus monkeys, synchronous, regular oscillations occurred in the plasma concentrations of glucose, insulin, and glucagon. The oscillations displayed a period averaging 9 minutes. The amplitudes for insulin and glucagon were ten and five times greater than for glucose. Insulin cycled in and glucagon out of phase with glucose. In baboons, oscillations of glucose and insulin were smaller than in rhesus monkeys, while in man, regular oscillations were not observed.*

While studying the changes that occur in circulating fuels and hormones during spontaneous feeding in rhesus monkeys (*Macaca mulatta*), we noted synchronous, regular oscillations in the concentrations of glucose, insulin, and glucagon in the plasma. The period of these oscillations was 7 to 12 minutes and remained relatively constant up to 1 hour in individual animals. The insulin and glucose cycles were nearly in phase while the glucagon cycles were out of phase with insulin and glucose. These observations were made in eight conscious monkeys that were restrained in chairs and had been prepared with venous cannulas permanently implanted in the vena cava at the level of the right atrium for blood sampling. The monkeys were fed on a complete liquid diet (Ensure, Ross) delivered by an automated feeder (*1*). The animals were housed in sound-attenuated chambers, their venous lines being led to the outside of the chamber so that samples could be taken without disturbing the monkey. Samples were drawn at 2- or 5-minute intervals for analyses (*2*) of glucose, insulin, and glucagon.

The cycles were studied in detail in six animals that had fasted for 16 hours. Figure 1 shows the data and time series analysis in one animal (No. 5, Table 1). Plasma was sampled every 2 minutes for 30 minutes in the morning before the first expected meal. The data were analyzed by autocorrelation with values being interpolated to 30-second intervals and periods of delay of (up to) 16 minutes (*3*). The period of the cycles for glucose, insulin, and glucagon did not differ by more than 1 minute (except in animal No. 4). The mean period for all animals was 9.3 ± 1.5 minutes (standard deviation, S.D.) (Table 1). The method of cross correlation was used to determine phase shifts. With glucose used as a reference, the maximum correlation with insulin occurred on an average 0.9 ± 0.6 minute later (or 8.4 minutes earlier) and for glucagon 6.8 ± 1.6 minutes later (2.5 minutes earlier) than glucose. Maximum correlation of the molar ratio of insulin to glucagon (I/G) occurred 1.0 ± 0.6 minute later (or 8.3 minutes earlier) than glucose.

The normalized autocorrelation coefficients for the variables are listed in Table 1. Although not every coefficient reached the level of significance in each animal, at least one of the variables displayed significant autocorrelation at the $P \lesssim .05$ level (in animal No. 6, I/G was the only variable to reach significance). The amplitudes of oscillation of the three variables were significantly different (insulin > glucagon > glucose) (Table 1). In these relatively short experiments the amplitude did not vary greatly from cycle to cycle in most animals. However, amplitudes did change in some time series and when this occurred the amplitudes of insulin and glucagon often changed in parallel.

The effect of a phantom meal was examined in three animals by filling the feeder and turning on the light, but not activating the diet delivery pump. Glucose and insulin cycled unchanged during and after this phantom meal. In contrast, when an authentic meal was eaten, the concentrations of glucose and insulin increased within 10 to 12 minutes and the cycles were interrupted. Atropine given intravenously (0.05 mg/kg) at the midpoint of a 60-minute period of observation did not disrupt the cycles of insulin

and glucose in two animals. In the postabsorptive state, the oscillations returned at 12 midnight in four animals last fed at 4 p.m. and then sampled at 8 p.m., 12 midnight, 4 a.m., and 8 a.m. We also observed two other species—the baboon (*Papio cynocephalus*) and humans. In baboons studied under similar circumstances, insulin and glucose exhibited cyclic behavior but with considerably lower amplitude and less consistency from cycle to cycle in both period and amplitude. The period was longer, averaging 13 minutes in three animals. In three normal humans studied with 2-minute sampling intervals for 2 hours after an overnight fast (basal conditions), regular oscillations were not observed. Glucose and insulin, however, did display intermittent synchronous fluctuations. The amplitude of oscillations when present was much smaller than that observed in the rhesus monkey.

As early as 1923, Hansen (*4*) described fluctuations in the blood sugar of fasting normal man at intervals of 5 to 10 minutes. Over the years a number of other investigators have reported oscillations of glucose or glucose and insulin in a variety of animals (*5–8*). Oscillations, when observed, have been irregular both in frequency and amplitude. The frequency of oscillations reported has varied greatly depending in large measure upon the frequency of sampling, as one might expect. Anderson *et al.* studying man (*5*) and dogs (*6*), and Iberall *et al.* studying man (*7*) have reported rapid oscillations of blood glucose with intervals of 30 to 40 seconds. Oscillations with longer intervals, 400 to 500 seconds per cycle, approximating the 9-minute interval reported here, were also noted by these investigators (*7*), although their sampling period of only 10 minutes precluded definitive observation of cycles with this or a longer interval. In a single dog (*6*) rapid oscillations of pancreatic venous insulin and hepatic venous glucose concentrations tended to show synchrony for about half of a 6-minute period of observation (sampling intervals of 15 to 30 seconds). Others have not been able to demonstrate significant oscillation of blood glucose (*9*) under basal conditions. The only regular oscillations of glucose and insulin previously reported occurred during glucose infusion in conscious fasted dogs (*8*). Glucose and insulin displayed regular, synchronous oscillations with a period of 50 minutes for up to 10 hours of constant glucose infusion. Careful observation of the basal state, however, led Ookhtens *et al.* (*8*) to conclude "that arterial glucose and insulin concentrations in fasted conscious dogs show no consistent oscillations." In retrospect, with the knowledge of the interval we have obtained, it is possible that a regular oscillation was intermittently present in their data. Other hormones display a fluctuating concentration in plasma. However, only luteinizing hormone (*10*) and growth hormone (*11*) appear to oscillate with a regular period shorter than the circadian period. The other pituitary hormones are characteristically secreted in episodic bursts without discernible regularity (*12*).

The obvious physiologic interactions of the three synchronously cycling variables suggests several hypotheses of the origin of the oscillations. The phase relationships between glucose, insulin, and glucagon in these time series fits their known stimulus-secretory relationships in the beta and alpha cells of the endocrine pancreas (islets of Langerhans). In addition, the observation that glucose leads insulin by a short interval is compatible with the possibility that a glucose

Table 1. Results of time series analysis by autocorrelation in eight *M. mulatta*. The concentrations of glucose, insulin (I), and glucagon (G) were measured in plasma sampled every 2 minutes for 32 minutes in animals 1 to 6. In animals 7 and 8, samples were collected every 5 minutes for 30 minutes, every 1 minute for 10 minutes, and then every 5 minutes for 20 minutes. The molar ratio, I/G, was analyzed in those animals assayed for glucagon. Listed for each variable are the means of all values in the time series and the average half amplitude (amp/2) about the mean, given as a percentage of the mean. The mean period (± S.D.) for each animal is the average of the time delays giving maximum positive correlation (r) for each variable on analysis by autocorrelation (see Fig. 1). Abbreviation: m/m, mole/mole.

Animal No.	Glucose			Insulin			Glucagon			I/G			Mean period (minutes)
	Mean (mg/dl)	Amp/2 (%)	r	Mean (μU/ml)	Amp/2 (%)	r	Mean (pg/ml)	Amp/2 (%)	r	Mean (m/m)	Amp/2 (%)	r	
1	69	2.6	.42	80	47	.78	168	15	.32	11.0	51	.28	8.6 ± 0.8
2	67	3.3	.60	77	28	.85	223	11	.50	8.1	33	.95	8.2 ± 0.3
3	71	4.9	.08	107	42	.88	149	15	.76	17.0	42	.96	8.3 ± 0.3
4	69	5.0	.58	68	30	.70	242	51	.46	7.0	53	.10	11.9 ± 1.9
5	65	5.6	.60	90	51	.95	205	16	.90	11.0	62	.95	10.2 ± 0.3
6	73	1.7	.20	46	22	.40	187	11	.25	5.7	28	.50	7.2 ± 0.7
7	65	4.9	.52	63	69	.49							9.9 ± 0.1
8	72	7.1	.60	44	121	.33							10.2 ± 0.2
Mean	69	4.4	.45	72	51	.67	196	20	.53	10.0	45	.62	9.3
S.D.	3.0	1.8	.20	21	32	.24	35	15	.25	4.1	13	.38	1.5

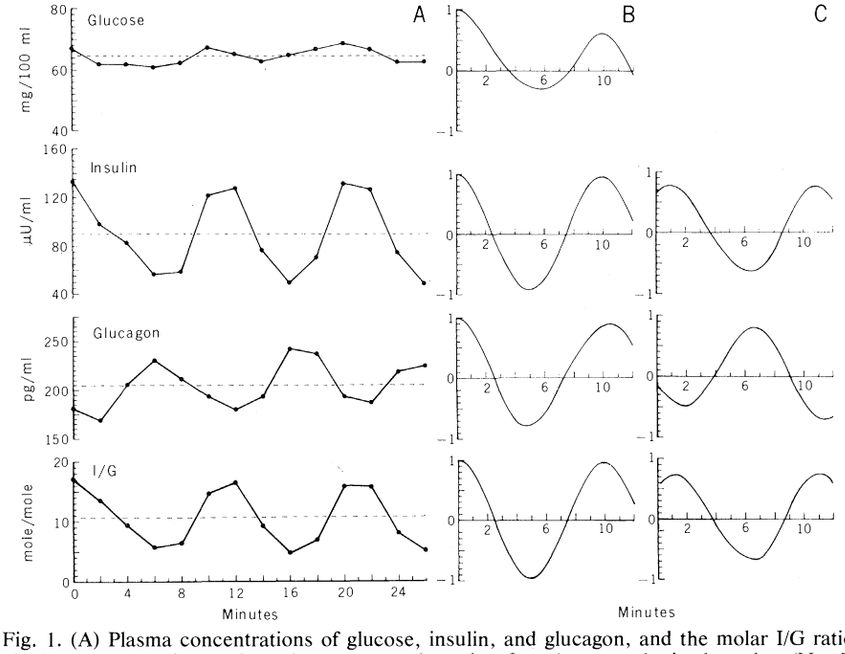

Fig. 1. (A) Plasma concentrations of glucose, insulin, and glucagon, and the molar I/G ratio obtained every 2 minutes through a venous catheter in a fasted, unanesthetized monkey (No. 5, Table 1). The dashed horizontal line indicates the mean value for each variable over the total sample period. (B) Normalized autocorrelation for each of the four variables. The time of the first local maximum (following time zero) was taken as the period of the oscillation, a value of 10 minutes for this animal. (C) Normalized cross correlation between glucose and the remaining three variables. The time of the first maximum correlation coefficient was taken as a measure of time delay referred to the glucose cycle. In this example, insulin, glucagon, and the I/G ratio exhibited delays of 1.0, 6.5, and 1.5 minutes, respectively.

cycle is driving the insulin (and glucagon) oscillation. It seems reasonable to postulate that the glucose cycle itself reflects cyclic output of glucose by the liver in these fasting animals, a conclusion supported by reported measurements of hepatic glucose production (*13*), although cyclic changes in peripheral glucose utilization have not been excluded. The interval of 0.9 minute between the glucose cycle and insulin cycle is sufficient to encompass recirculation of glucose from the hepatic veins to the pancreatic artery plus time for delays in stimulus-secretion coupling in the islets (*14*). The phase relationships between cycles are appropriate for a linear feedback system with time delay, involving glucose and the endocrine pancreas. However, as Ookhtens *et al.* (*8*) point out, such a simple linear oscillator is inherently unlikely, and a nonlinear system is the better possibility.

The cells of origin of all three components displaying oscillatory behavior (the liver, and the beta and alpha cells of the islets) possess autonomic neural connections and are, at least in part, controlled by the central nervous system (*15*). Accordingly, cyclic stimulation of any or all components can be transmitted from an oscillator in the central nervous system. Since the cycles are not interrupted by atropine, neural transmission would not appear to depend upon parasympathetic innervation.

Another possibility is suggested by the recent demonstration of gap junctions between adjacent islet cells (*16*), the islet cells probable embryonic origin from neural crest, and the observation of spontaneous electrical activity in islet cells (*17*). These observations have suggested the hypothesis that the cells within the islets of Langerhans may function as an integrated unit under certain physiologic circumstances. This hypothesis has been extended to include a role for the D cells in the islets of Langerhans shown recently to contain the secretory inhibitor, somatostatin (*18*). All of these considerations taken together suggest that the observed oscillations in insulin and glucagon could originate with spontaneous fluctuations in activity within the islet complex; however, such a mechanism would require interislet communication.

A central nervous system origin for the oscillations would be greatly supported by finding other oscillations of the autonomic nervous system with which the observed oscillations were entrained. However, examinations of recordings made in rhesus monkeys of heart rate, blood pressure, respiration, skin temperature, and gastric motility failed to uncover cyclic phenomenon of comparable frequency. Further study will be necessary to place these observations in proper physiologic perspective. Our studies indicate that the rhesus monkey is particularly suited for study of this phenomenon, but it seems unlikely that the cyclic behavior of glucose, insulin, and glucagon will prove to be unique to this species.

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Interaction of *Beggiatoa* and Rice Plant: Detoxification of Hydrogen Sulfide in the Rice Rhizosphere

Abstract. Beggiatoa *was obtained from six habitats, including four water-saturated soils from rice fields. The isolate of* Beggiatoa *from Bernard clay, when reinoculated into soil treatments from pure culture, significantly reduced hydrogen sulfide levels in soils and increased oxygen release from rice plants. Rice plants significantly increased* Beggiatoa *survival in flooded soils. Some hydrogen sulfide was necessary for survival of the Bernard clay isolate; high concentrations of hydrogen sulfide killed the Bernard clay isolate but were tolerated by a Crowley silt loam isolate from Eagle Lake, Texas. The results suggest that* Beggiatoa *may be an element of wetlands plant ecosystems.*

Pitts *et al.* (*1*) suggested a mutually favorable interaction between the filamentous (gliding) bacterium *Beggiatoa* (*2*) and rice plant. A more general aspect of the physiology and nutrition of the genus *Beggiatoa* and its distribution in soil and water has arisen from studies by Pringsheim and co-workers (*3*). We have shown: (i) that hydrogen sulfide (H_2S) inhibits oxygen release from, and nutrient uptake by, rice plants; and (ii) a correlation between responses of rice cultivars to H_2S and sulfide diseases (*4*, *5*). In this report we describe the mutual interdependence of *Beggiatoa* and rice seedlings exposed to H_2S, as well as specific instances of protection of rice seedlings from H_2S by *Beggiatoa*.

Three types of soil obtained from a greenhouse (GH), the Eagle Lake area in Texas (Crowley silt loam, CSL), and south Louisiana (Bernard clay, BC) were air-dried to kill any indigenous *Beggiatoa*. We placed 300 g of each soil (moist weight) separately into 21 Mason jars (capacity, 946 ml each). These jars were then grouped into seven sets of three jars each. The jars in sets 1, 2, 3, and 4 received sufficient sterile tap water to produce a layer 3.5 cm thick on the soil surface; jars in sets 2, 4, 5, and 7 received approximately 700 trichomes per gram of soil of a pure culture isolate of *Beggiatoa* obtained originally from BC and maintained on agar medium. All jars were then sealed with their lids and placed