mer, $(S)_C(R)_P$, should be enzymatically hydrolyzed least rapidly.

The evidence that there is some enzymatic hydrolysis of this isomer, albeit more slowly, suggested that a longer exposure to squid-type DFPase would result in more nearly complete hydrolysis. Table 1(B) shows that a $10^{-3}M$ solution of Soman is almost completely hydrolyzed by a 5 by 150 mm agarose-DFPase column with a flow rate of 0.067 ml/min and a time until fluoride appearance of 45 minutes. It can also be seen that now the extent of hydrolysis is virtually the same whether measured by the production of fluoride or by the loss of AChE-inhibiting power.

In addition to confirming that the $(R)_P$ pair of phosphorus-centered isomers of Soman is a much more potent AChE inhibitor than the other, our results present the disturbing evidence that this pair is also the least rapidly enzymatically detoxified. Nevertheless, enzymatic detoxication can be accomplished by a longer exposure to the hydrolyzing enzyme. Although the use of DFPase from such a relatively limited source as the squid, bound to such a fragile support as agarose, is probably not practical for the large-scale detoxication of unwanted nerve gas, the potential exists for accomplishing this by a combination of the techniques of genetic manipulation (*17*) and biochemical engineering (*18*).

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Hepatic Glucose Production Oscillates in Synchrony with the Islet Secretory Cycle in Fasting Rhesus Monkeys

Abstract. *Oscillations in the concentration of plasma glucose were found to reflect large fluctuations in hepatic glucose production. The fluctuations in glucose production were synchronous with fluctuations in the concentration of plasma insulin and glucagon. This synchrony suggests that hepatic pathways are entrained to the islet cycle with a minimal time delay.*

Insulin and glucagon are secreted as regular synchronous pulses in nonhuman primates that have fasted overnight (*1*). These hormonal cycles are accompanied by smaller synchronous oscillations in the plasma glucose concentration. We have investigated the origin of the glucose fluctuations in unanesthetized rhesus monkeys (*Macaca mulata*) prepared for measurement of splanchnic glucose production by the method of Bowden and Bergman (*2*). We postulated that the glucose cycle originates in cyclic hepatic glucose production because, in the fasting state, the hepatic actions of the islet hormones predominate over peripheral actions. The phase relations between the glucose cycle and the hormonal signals

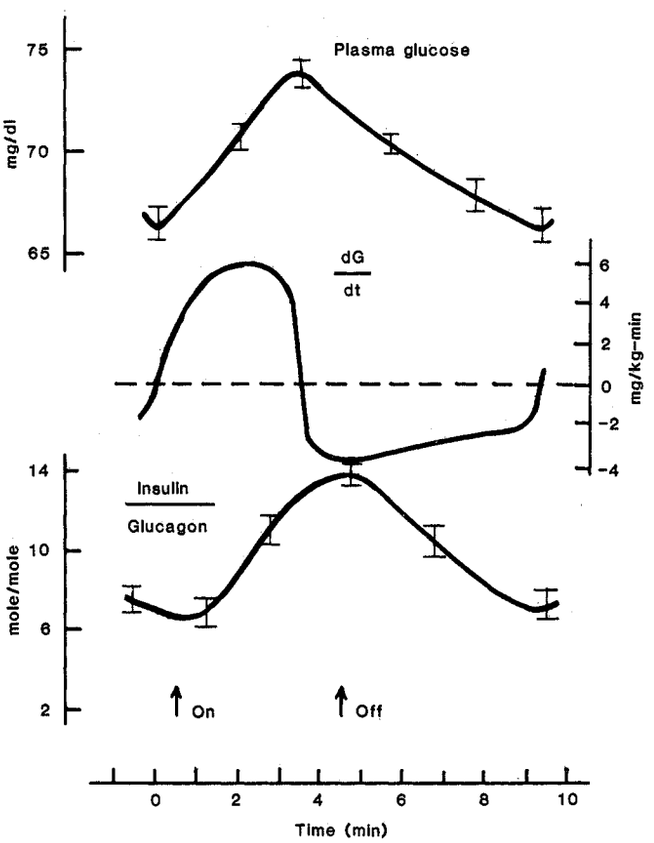

Fig. 1. The plasma glucose cycle compared with the cycles for insulin and glucagon, here combined into the insulin, glucagon molar ratio. The waveform for the plasma glucose cycle was derived by analysis of 94 separate glucose cycles from 48 experiments in 11 rhesus monkeys displaying significant glucose oscillations. The derivative of the glucose concentration cycle was used to calculate the rate of change of glucose in the exchangeable glucose pool (G) which occurs each cycle. This parameter, dG/dt, has been plotted in correct phase relation to the insulin, glucagon molar ratio. The arrows indicate points of maximum on or off signal to hepatic pathways for glucose production. The behavior of the bihormonal signal and the predicted change in entry of glucose into the glucose space correspond closely in the time domain.

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strongly favor this hypothesis. These relations are summarized in Fig. 1, in which the average waveforms (*3*) for the glucose cycle and for the insulin and glucagon cycles (presented as the molar ratio) are plotted according to their mean phase relation as determined by cross-correlation of individual time series (*4*) in nine overnight-fasted rhesus monkeys. Because glucagon characteristically oscillates with a 5- to 6-minute delay relative to insulin during cycles averaging 9 to 10 minutes in length, the net signal to hepatic metabolic pathways is an amplified on and off message each cycle. The maximum on and off signals to hepatic glucose production (indicated by arrows) occur at the beginning of the upstroke and downstroke, respectively, of the plasma glucose cycle. To estimate the excursions of hepatic glucose production necessary to produce the plasma glucose cycle, we calculated the rate of change in the rapidly mixing pool of glucose expected from the rate of change in plasma glucose concentration and the estimated size of the glucose space (*5*). This estimated parameter (dG/dt, Fig. 1) shows an early increase to positive values above 6 mg per kilogram per minute followed by a more prolonged period of negative values of smaller magnitude. The timing of the positive and negative phases also corresponds closely to the predicted hepatic activity of the bihormonal signal.

Hepatic glucose production was estimated every 2 minutes for 30 minutes in five consecutive experiments in a 4.8-kg rhesus monkey adapted to chair restraint and fasted overnight. Hepatic plasma flow as measured with indocyanin green did not display significant fluctuations or regular oscillations and averaged 31 ± 6 ml/kg-min (mean ± standard error). Hepatic glucose production was estimated by multiplying the difference between hepatic venous and arterial glucose concentrations, determined every 2 minutes, by hepatic plasma flow. These data are plotted against time in Fig. 2A together with the simultaneously measured concentrations of arterial plasma insulin. In all five experiments arterial plasma glucose and insulin displayed significant regular oscillations by autocorrelation, $P < .05$ (mean period of oscillation, 9.2 ± 0.8 minutes). Hepatic glucose production displayed significant autocorrelation in experiments 2 and 5, $P < .01$ (period 9.5, 8.3 minutes) and significant cross-correlation with plasma insulin concentration in experiments 1, 2, 3, and 4, $P < .05$. For these four experiments, the mean delay between the insulin cycle and hepatic glucose production was 5.1 ± 0.90 minutes.

To test the significance of the apparent cycle in hepatic glucose production and to further establish its synchrony with the insulin cycle, we used the method of standard array averaging as described by Lang and co-workers (*6*). This method is based on the summation of events coincident in time and separates those events which occur randomly (noise) from those which occur synchronously with an endogenous cycle (insulin). The method is

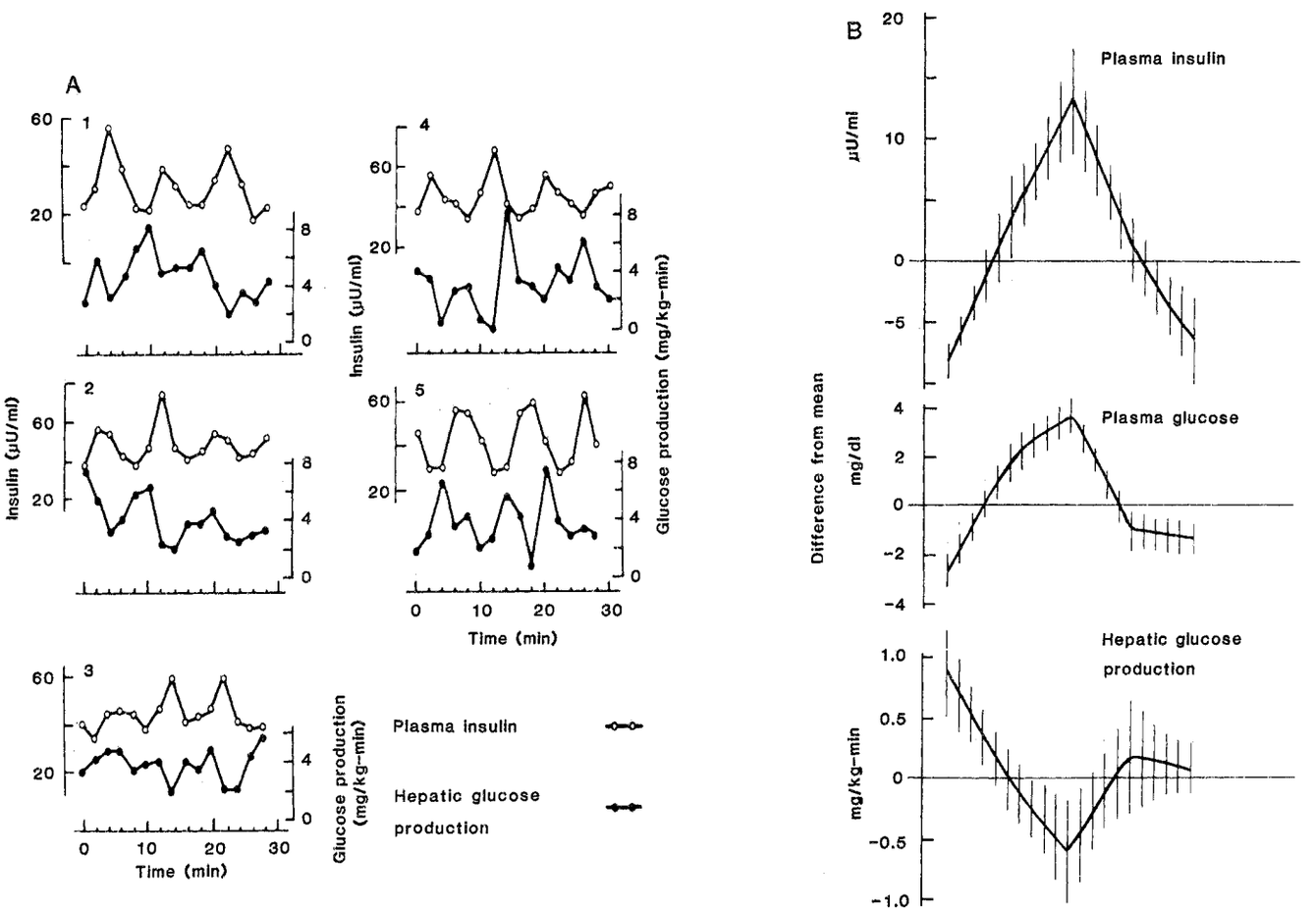

Fig. 2. (A) Five consecutive experiments in a rhesus monkey prepared for sampling mixed hepatic venous plasma. The calculated net hepatic (splanchnic) glucose production measured every 2 minutes is plotted in comparison to the concentration of insulin in arterial plasma. The animal was studied after an overnight fast in the unanesthetized state. (B) An averaged array of the 15 insulin cycles from five experiments was used to generate time-coincident arrays for plasma glucose concentration and hepatic glucose production. Each cycle was interpolated into 20 equal intervals, the means and standard errors were calculated, and the data plotted as increments from the mean for each cycle. In each case the curves for the averaged cycle differ significantly from zero.

analogous to techniques used in analyzing evoked potentials in neurophysiologic studies (*7*). Figure 2B shows that plasma glucose and hepatic glucose production display significant time-dependent changes in synchrony with the insulin cycles. The timing of these cycles closely matches the predicted relations illustrated in Fig. 1. The apparent amplitude of the cycles is reduced by this method of analysis because of the averaging of cycles with slightly different phase relations. When the amplitudes of the excursions of hepatic glucose production were analyzed for each experiment according to standard criteria, they ranged from 39 to 92 percent of the mean. For the five experiments, the mean hepatic glucose production was 4.1 ± 0.4 mg/kg-min and the mean amplitude was 59 ± 10 percent of the mean. To confirm the validity of the directly measured glucose production, we also estimated the rate of glucose production on three separate days using [3-$^3H_1$]glucose by the constant infusion method (*5*). The rate averaged 4.3 ± 0.3 mg/kg-min, which is in excellent agreement with the mean rate directly determined.

In the overnight fasted animal, hepatic glucose production depends largely on glycogenolysis, but as the period of starvation increases, the contribution of gluconeogenesis increases. Insulin and glucagon regulate the rates of both processes through opposing actions (*8*). Hepatic glycogen synthase and phosphorylase respond very rapidly to single doses of insulin or glucagon in vitro and in vivo (*9*). The time constants for hormonal control of gluconeogenesis may be considerably longer, particularly for the decay of insulin-induced inhibition in vivo (*10*). In this study hepatic glucose showed cycles of low and high rates of production but did not display excursions into the range of net glucose uptake. These data are compatible with a cycle based on acceleration and deceleration of the glycogenolytic pathway, a pattern that could be induced by cyclic glucagon alone. However, because phosphorylase and glycogen synthase are reciprocally regulated by both hormones, both steps probably undergo some degree of cyclic activation and deactivation with the net rate favoring glycogenolysis throughout the cycle. The relative contribution of each hormone to entrainment of these pathways cannot be determined from this study, but the close correspondence between the observed events and the predicted response to the combined signal leads us to favor the view that both hormones are important for coordinated control.

If our hypothesis is true, control of the phase relation between the secretory cycle of the A and B cells becomes a potentially important mode of regulation for the hepatic response and for insulin delivery to the periphery. For example, if insulin and glucagon were to be secreted in phase, their activities would tend to cancel rather than amplify each other, and one would predict a smaller amplitude in entrained hepatic glucose production. Furthermore, large peaks of glucagon secreted in phase with insulin might permit correspondingly larger secretory peaks of insulin to be transmitted to the periphery without changing hepatic glucose production. Since the time constants for peripheral insulin effects (*11*) are probably longer than for hepatic effects, the net result would be preservation of hepatic glucose production during heightened insulin responses in the periphery, for example, during assimilation of a protein meal (*8*). The demonstration (*12*) that under conditions of intravenous glucose loading, cycles of insulin-mediated peripheral glucose utilization show higher amplitude and lower frequency (period 90 minutes) also suggests the possibility that frequency modulation may be important under some conditions.

The physiologic and pathophysiologic significance of the cyclic mode of control remains to be established. We postulate that two general functional characteristics might be influenced by the synchronization of hepatic metabolic pathways to oscillating insulin and glucagon: the hormonal dose-response characteristics and the efficiency of metabolic responses to changing physiologic demands. With regard to hormone dose-response characteristics, it has been shown that the effectiveness of glucagon given by continuous infusion decreases in time and is restored by interposition of a recovery period (*13*). Further, when glucagon is withdrawn from the portal circulation for a longer period, the response to reinfusion of glucagon is also diminished (*14*) and returns to normal only after a suitable period of exposure to the hormone. We postulate, therefore, that the cyclic exposure of hepatocytes to glucagon normally conditions optimal hormone responses in a manner analogous to the dependence of normal gonadotropin secretion upon cyclic exposure to luteinizing hormone–releasing hormone (*15*).

With regard to enhanced metabolic efficiency, it has been postulated on theoretical grounds that cyclic operation of catabolic pathways may increase the efficiency of conversion of precursor to product (*16*) and that cyclic operation of the glycolytic pathway reduces the dissipation of free energy (*17*). The intrinsic cellular oscillations of glycolysis which characterize most cells (*18*) probably also occur in hepatocytes and become entrained to the islet cycle. Whether such entrainment may contribute to the overall efficiency of metabolic pathways in vivo requires investigation. Our data also suggest that the rate of hydrolysis of the outer tier of glycogen undergoes a marked change each cycle. If actual glycogen synthesis also occurs each cycle, the net effect would be analogous to the operation of a futile substrate cycle, for example, the recycling of glucose through glucokinase and glucose-6-phosphatase. Newsholme (*19*) has discussed the theoretical advantages in enhanced flexibility of response which accompany such cycles.

Among the events that might be optimally conditioned by the mode of hormone secretion are the rate of response in glycogenolysis to sudden increments in signal, the overall rate of depletion of the glycogen store during the daily feeding and fasting cycle, and the relative activity of the other hormone-sensitive hepatic pathways, that is, gluconeogenesis, ketogenesis, pyruvate oxidation, and lipogenesis.

If the cyclic mode of hormone secretion proves to have biologic advantage for regulating hepatic metabolism, the possibility that derangements in the time domain contribute to pathophysiology in metabolic disease will warrant consideration.

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Synapses Between Neurons Regenerate Accurately After Destruction of Ensheathing Glial Cells in the Leech

Abstract. *Individual glial cells that ensheathe axons in the central nervous system of the leech were destroyed by intracellular injection of protease. The axons were then severed, and regeneration by particular neurons was studied physiologically and morphologically. Although certain axons sprouted more in the absence of the glial cell, functional synapses were accurately regenerated with normal frequency.*

It has been proposed that the glial cells that surround neurons in vertebrates and invertebrates play a variety of roles in nerve regeneration. For example, glia may guide regenerating axons in the central nervous system (CNS) of lower vertebrates (*1*) and in the peripheral nervous system of all vertebrates (*2*). On the other hand, glia may interfere with axon growth in the mammalian CNS (*3*), where regeneration occurs only to a limited extent. Invertebrate systems are particularly useful in the study of nerve regeneration, since the regeneration is accurate and can be examined at the level of individual cells (*4*). However, the involvement of glia in regeneration in invertebrate systems has not been established.

The CNS of the leech contains large glial cells and hence offers an opportunity to test directly the role of glia in nerve regeneration. After nerve injury in the leech CNS, the glial cell sheath survives; the glia are thus candidates for guiding regenerating axons (*5*). In the present study, we destroyed individual glial cells in the CNS of the leech *Hirudo medicinalis* by intracellular injection of a protease and observed the regeneration of axons and synapses in the absence of the ensheathing glial cell. While the growth pattern of regenerating axons was atypical in some cases, the axons still reestablished correct synaptic connections.

The leech CNS consists of a chain of ganglia which contain nerve cell bodies and synaptic neuropil and which are linked by three axon bundles called connectives. Each lateral connective, containing several thousand axons, is ensheathed between any two ganglia by a single glial cell several millimeters long. The medial connective, containing about 100 axons, is usually ensheathed along part of its length by one of the two connective glial cells and along the remainder of its length by the other glial cell.

One or both of the connective glial cells were killed by pressure injection into the cell of protease (Subtilopeptidase A, type VIII, Sigma; 6 mg/ml in 0.2*M* KCl containing 0.2 percent fast green FCF) through beveled recording electrodes (*6*). By observing the coloration of the glial cell, we could control the injection and destroy the glial cell without measurably affecting the axons it surrounded (Fig. 1, A and B). Destruction of the glial cell was verified by light microscopy of 2-μm-thick sections and by electron microscopy of ultrathin sections (*5, 7*). Axon health was monitored by physiological tests of impulse conduction and by electron microscopy. Glial cell killing resulted in an increase in the number of microglia (Fig. 1B), macrophage-like cells which exist in the normal leech nerve cord (*8*) and in greater numbers in the injured nerve cord (*5*). As judged from their appearance under the electron microscope, these cells participated in the removal of glial debris and in time ensheathed portions of some axons (Fig. 1C). In general, protease-injected glia were gone after 1 week, at which time the axons of the connectives were severed near one ganglion by crushing the correctives with fine forceps (*5, 7*). At various times during the ensuing 24 weeks the preparations were dissected from the animal and examined for regeneration (*5, 7*).

Regeneration by interneurons and mechanosensory neurons was studied. The S cell, an interneuron that occurs singly in each ganglion, projects an anterior and a posterior axon in the medial connective. Each S axon forms an electrical synapse near its tip midway along the connective with the axon of the S cell in the adjacent ganglion (*7, 9*). The two pressure (P) and two nociceptive (N) mechanosensory neurons on each side of every ganglion project axons in the ipsilateral connectives and form chemical synapses with motor neurons in neighboring ganglia (*4*). With glia present, both the S axon and the sensory cell axons can regenerate after a lesion of the connectives and reestablish correct synaptic connections (*7, 10*).

To test physiologically for regeneration of the S axon in the absence of glia, we recorded simultaneously with intracellular electrodes from the axotomized S cell and its target S cell in the adjacent ganglion. Synapse regeneration, evidenced by electrical coupling between the two S cells (Fig. 2A), occurred in all 12 preparations tested 4 weeks or more after injury. This is comparable to the frequency of S cell regeneration found previously in the presence of the glial sheath (*7*).

The physiological test for regeneration of P and N cells involved stimulating the cell body in the ganglion anterior to the injected glial cell and recording from the ipsilateral L motor neuron in the adjacent posterior ganglion. The L cell innervates longitudinal muscle and is one of the normal synaptic targets of P and N cells. Successful regeneration, evidenced by a unitary synaptic potential in

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