Oscilaciones glicolíticas

•Observadas en levadura a fines de la década del 50. (organismo anaeróbico)

•Requieren una inyección permanente de sustrato (¿de cuál?)

•Oscilaciones: existen en un rango de valores de inyección: hay dos valores críticos en los que desaparecen

Aparecen si se inyecta:
glucosa 6-fosfato o
fructosa 6-fosfato

Sustancias involucradas en los pasos previos a los catalizados por la PFK

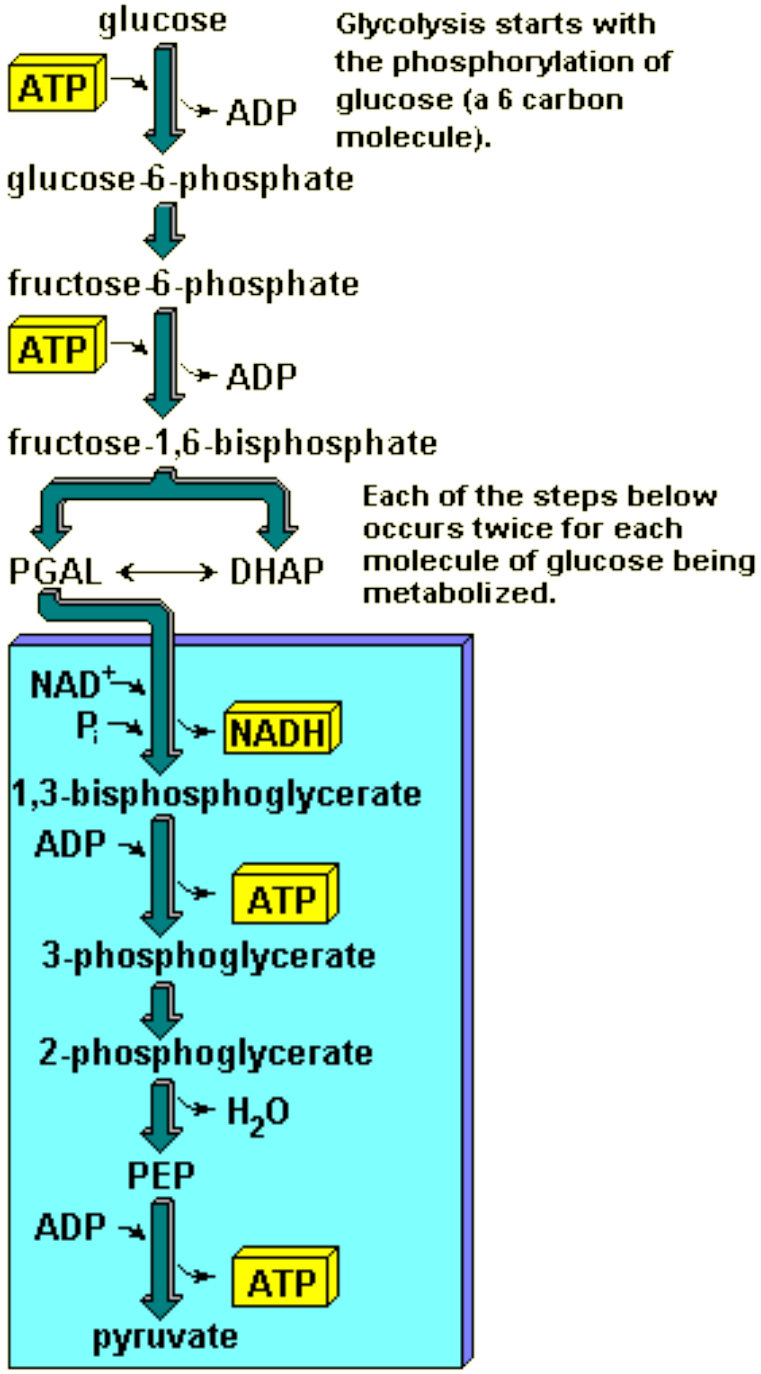
No aparecen si se inyecta:
fructosa 1,6 bifosfato

Sustancia involucrada en los pasos posteriores a los catalizados por la PFK

La PFK cumple un rol importante, ya que las oscilaciones desaparecen si se la inhibe

Hexokinase - phosphorylates glucose trapping it in the cell

Phosphofructokinase (PFK) – sets molecule up for cleavage



Se observan mirando la fluorescencia del NADH
(El NADH absorbe luz de 340 nm y emite a 445 nm.)

La primera observación (Duysens and Amesz, 1957) fue la de una oscilación que se amortiguaba rápidamente.

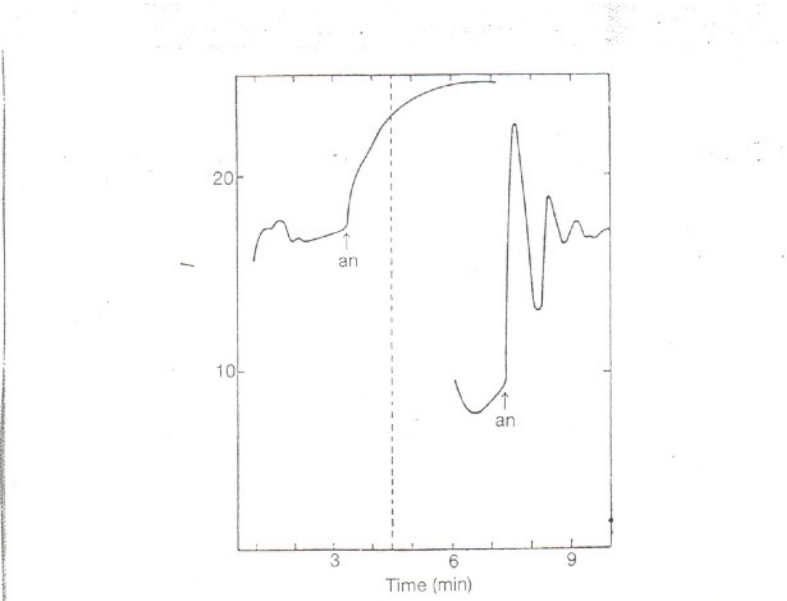


Fig. 2.1. Damped oscillations in the fluorescence of a glycolytic intermediate, NADH, following the injection of glucose (right) in a suspension of yeast cells. This observation was the first indication of the possibility of oscillatory behaviour in glycolysis. The curve on the left shows the addition of ethanol. an, anaerobic condition (Duysens & Amesz, 1957).

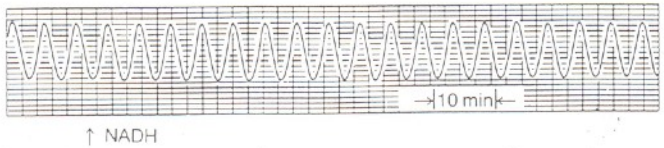
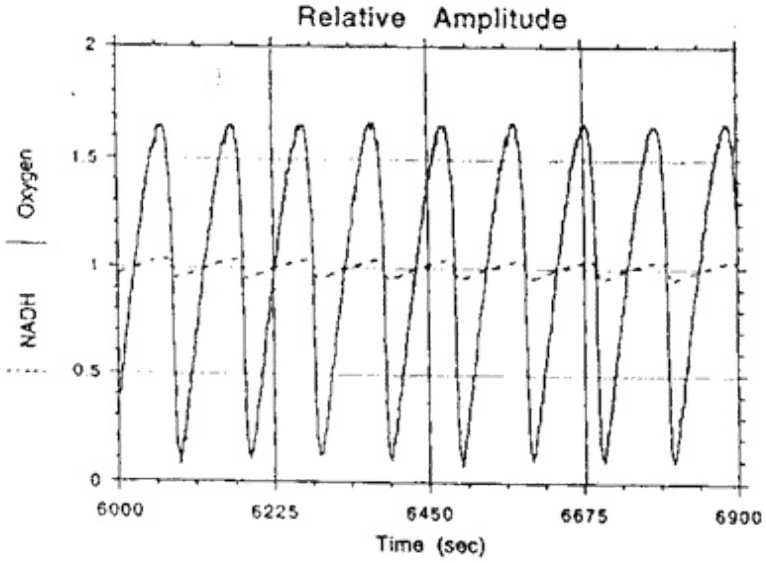


Fig. 2.2. Sustained oscillations in an extract of the yeast *Saccharomyces carlsbergensis* utilizing trehalose as the glycolytic substrate. The slow degradation of this substrate gives rise to regular oscillations that can be maintained for more than 100 cycles (Pye, 1971). The oscillations are recorded by measuring the fluorescence of the glycolytic intermediate, NADH.

Posteriormente se pudieron ver oscilaciones más sostenidas



No sólo se vieron oscilaciones en conjuntos de células, sino también en células solas.

Todos los intermediarios oscilan con igual frecuencia y, en general, distinta fase.

Las oscilaciones se observan para un cierto rango de tasas de inyección. La desaparición de las oscilaciones es reversible.

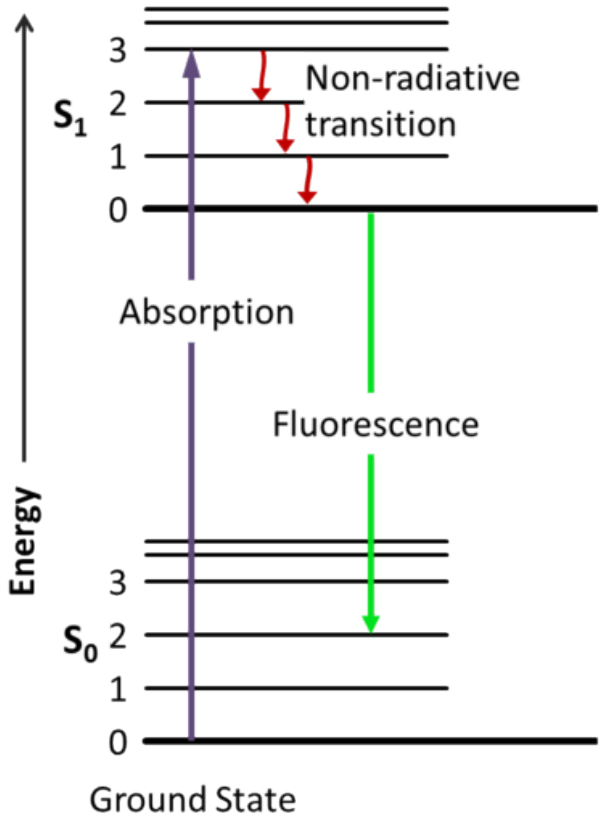
Se observaron tanto en extractos como en suspensiones de células intactas.

Input rate of fructose or glucose (mM/ h)	Period (min)	Amplitude (mM NADH)	Damping	Waveform
<20		Steady high level of NADH		
20	8.6	0.2-0.4		Double periodicities, nonsinusoidal
40	6.5	0.6		Nonsinus-sinus
60-80	5.0	0.3		Sinus
120	3.5	0.2		Sinus
>160		Steady high level of NADH	+++	

Recordemos, fluorescencia

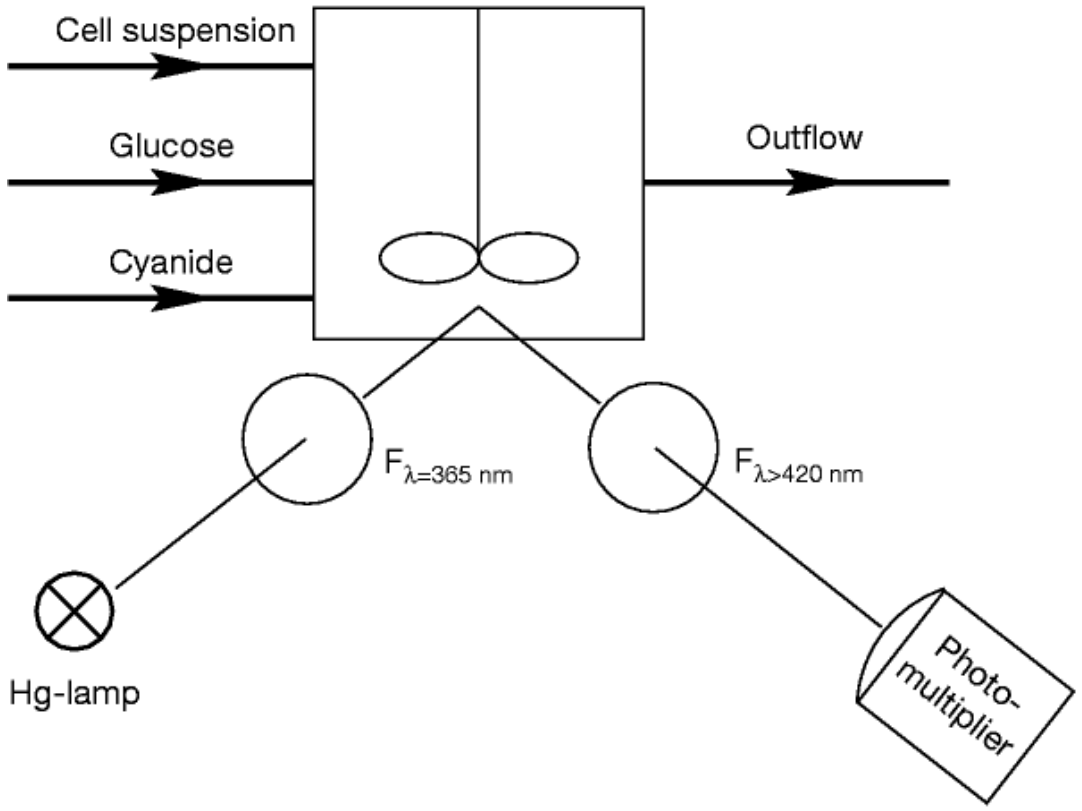
Es la emisión de luz por parte de una sustancia que absorbió luz. En la mayoría de los casos la longitud de onda de la luz emitida es mayor (o sea, de menor energía) que la de la absorbida.

Electronic transitions are about 1 eV. Vibrational transitions are about 0.1 eV. Rotational transitions (not shown) are about 0.001 eV. Absorption is about 1 femtosecond, relaxation takes about 1 picosecond, fluorescence takes about 1 nanosecond.
S0 and S1 represent different electronic states. The other numbers (here 0–3 are shown) represent vibrational states.
(Wikipedia).



Observación de oscilaciones por tiempo indefinido:

SUNE DANØ, PREBEN GRAAE SØRENSEN & FINN HYNNE
Sustained oscillations in living cells , *Nature* **402**, 320 - 322 (1999)



Las células están vivas pero en estado de “inanición”. En este caso también usan una suspensión de células de levadura.

Sustained oscillations in living cells

SUNE DANØ, PREBEN GRAAE SØRENSEN & FINN HYNNE

Nature **402**, 320 - 322 (1999)

Glycolytic oscillations in yeast have been studied for many years simply by adding a glucose pulse to a suspension of cells and measuring the resulting transient oscillations of NADH. Here we show, using a suspension of yeast cells, that living cells can be kept in a well defined oscillating state indefinitely when starved cells, glucose and cyanide are pumped into a cuvette with outflow of surplus liquid. Our results show that the transitions between stationary and oscillatory behaviour are uniquely described mathematically by the Hopf bifurcation. This result characterizes the dynamical properties close to the transition point. Our perturbation experiments show that the cells remain strongly coupled very close to the transition. Therefore, the transition takes place in each of the cells and is not a desynchronization phenomenon. With these two observations, a study of the kinetic details of glycolysis, as it actually takes place in a living cell, is possible using experiments designed in the framework of nonlinear dynamics.

Se ven o no se ven oscilaciones dependiendo de la tasa de inyección

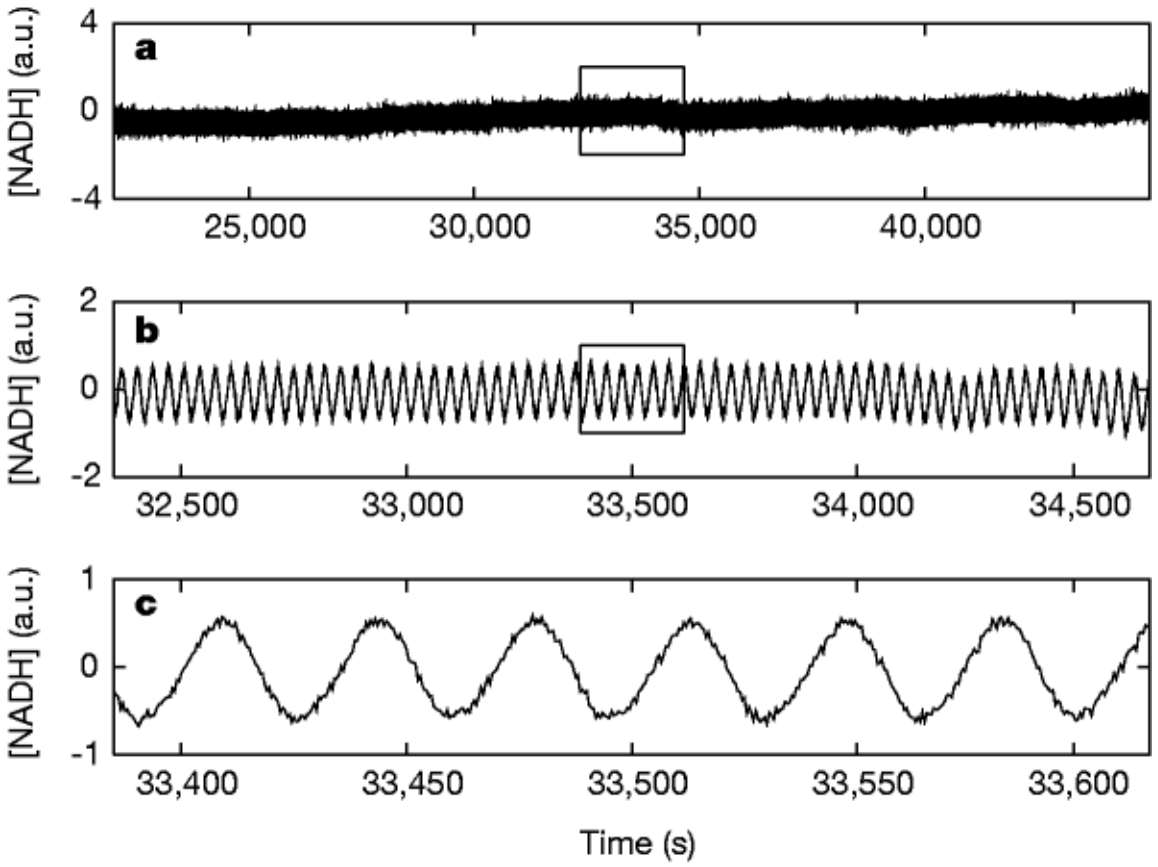


Figure 2 Sustained oscillations in *Saccharomyces cerevisiae*. **a**, An unperturbed part of a CSTR experiment. The period of the oscillations is around 34.8 s, and the amplitude is constant with a relative standard deviation of 2.8%. **b**, An expansion of the box in the top panel. **c**, A similar expansion of the box in the middle panel. The cell density is 1.61 10^9 cells per ml and the specific flow rate is 50.6 x10^{-3} min^{-1}. The mixed flow concentrations of glucose and cyanide are 35.0 mM and 5.38 mM. The experiment starts at time zero. a.u., arbitrary units.

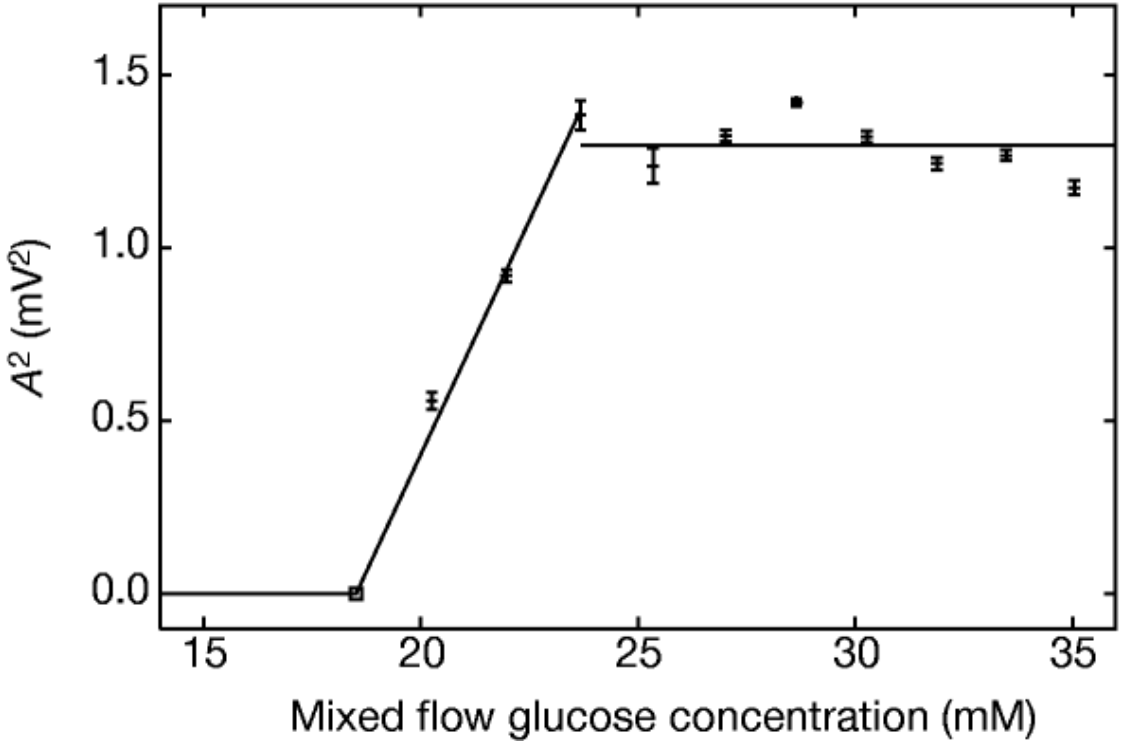


Figure 3 Plot of the square of the amplitude *A* as a function of glucose flow rate. The bifurcation point is found at a mixed flow glucose concentration of 18.5 mM. Oscillations were sinusoidal throughout the whole range of measurements. Cell density, mixed flow cyanide concentration and specific flow rate were fixed at 1.61×10^9 cells per ml, 5.60 mM and 4.86 10^{-3} min^{-1}, respectively.

También se observa una bifurcación similar cuando se varía la tasa de inyección del cianuro. En este caso hay dos bifurcaciones.

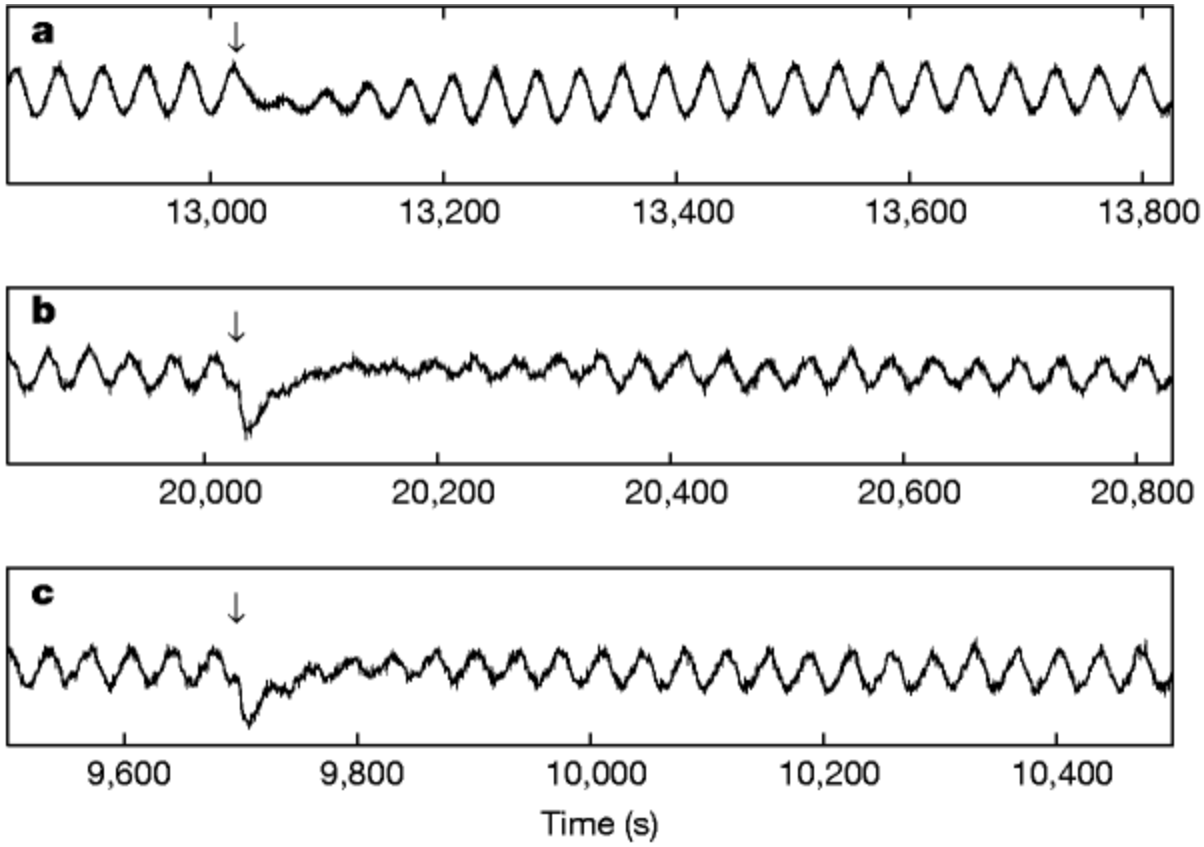


Figure 4 Fluorescence traces showing the response to instantaneous addition of extracellular glucose or acetaldehyde. **a**, Addition of glucose at a phase of 4° (arrow) with a change in glucose concentration of 1.11 mM, which quenches the oscillations. **b**, Quenching with 98 μM acetaldehyde at 172° (arrow). The uniqueness of the quenching phase and concentration change is illustrated in **c**, which repeats the experiment in **b** exactly, except that the phase is 180° (arrow) instead of 172°: the amplitude is diminished, but the oscillations do not stop completely.

Detailed understanding of the oscillations in glycolysis and their synchronization may be relevant to other cell systems. Glycolytic oscillations have been reported in many other cell types, notably muscle cells pancreatic -cells and heart cells. The possible coupling of the glycolytic oscillator to other cellular processes through oscillations in the [ATP]/[ADP] ratio might be important for the pulsatory insulin secretion of -cells or in the spatio-temporal regulation of blood flow. Furthermore, a synchronization mechanism, similar to the one seen in our experiments, could work through the blood as well. This could explain the existence of bulk oscillations of insulin concentration in the blood.

Yeast cells are basically unicellular organisms when in their natural environment. Nevertheless, the glycolytic clock provides a hidden potential for oscillatory communication. In our experiments the clock is awakened by the unnatural environmental conditions imposed by the special harvesting and addition of cyanide. From the theory of dynamical systems, it is known that a Hopf bifurcation is not a singular point in parameter space, but implies an infinity of Hopf points on a 'surface' possibly extending to conditions which might arise spontaneously in a changing natural environment. Thus, our experiments suggest an evolutionary path from unicellular to multicellular behaviour.

El rol de la PFK en las oscilaciones es corroborado por el hecho de que las mismas se ven afectadas cuando se agregan inhibidores o activadores de la enzima.

¿Por qué es importante la PFK para que ocurran las oscilaciones?

Porque su actividad (su capacidad catalizadora) es “efectivamente” modulada por un producto de la reacción, el ADP (también por el AMP)

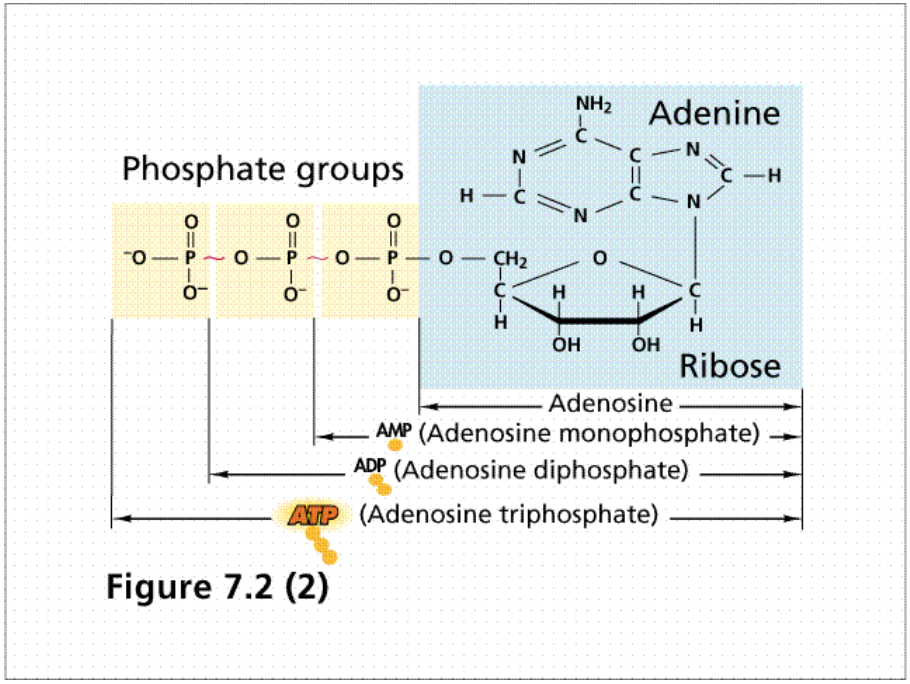


Figure 7.2 (2)

Más aún, el ADP “activa” la enzima (es decir, hay un “feedback” positivo!)

Es muy raro que un producto active una enzima. En este caso ocurre porque se trata de un paso en una secuencia de reacciones cuyo producto final es el ATP y no el ADP.

Las enzimas involucradas en sistemas de reacciones que consumen ATP son activadas por ATP e inhibidas por un exceso de ADP y AMP. Las involucradas en los caminos de reaccion que regeneran el ATP son inhibidas por un exceso de ATP y activadas por AMP y ADP.

Feedback positivo: autocatálisis

Autocatálisis + remoción + inyección → Puede dar lugar a oscilaciones

El control que ejerce el ADP se observa agregando ADP durante la oscilación, lo que produce un cambio abrupto en la fase de la oscilación.

Modelo de Selkov

Modela sólo un paso de esta cadena + la activación de la enzima por parte del ADP.

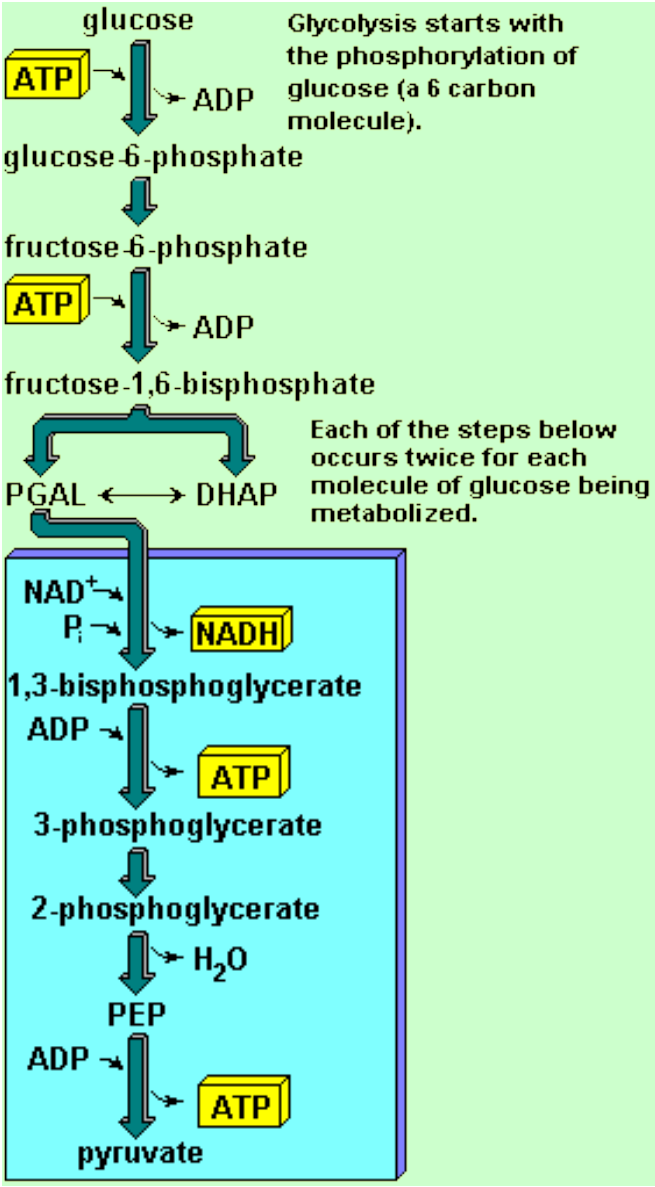
Las variables deberían ser [ATP], [ADP], [F6P], [FBP]. Como cambios de [F6P] y [FBP] no producen efectos notables, se consideran sólo al [ATP], [ADP], la enzima y el complejo.

$$\xrightarrow{\nu_1} S_1 + ES_2^{\gamma} \underset{k_{-1}}{\overset{k_{+1}}{\leftrightarrow}} S_1ES_2^{\gamma},$$

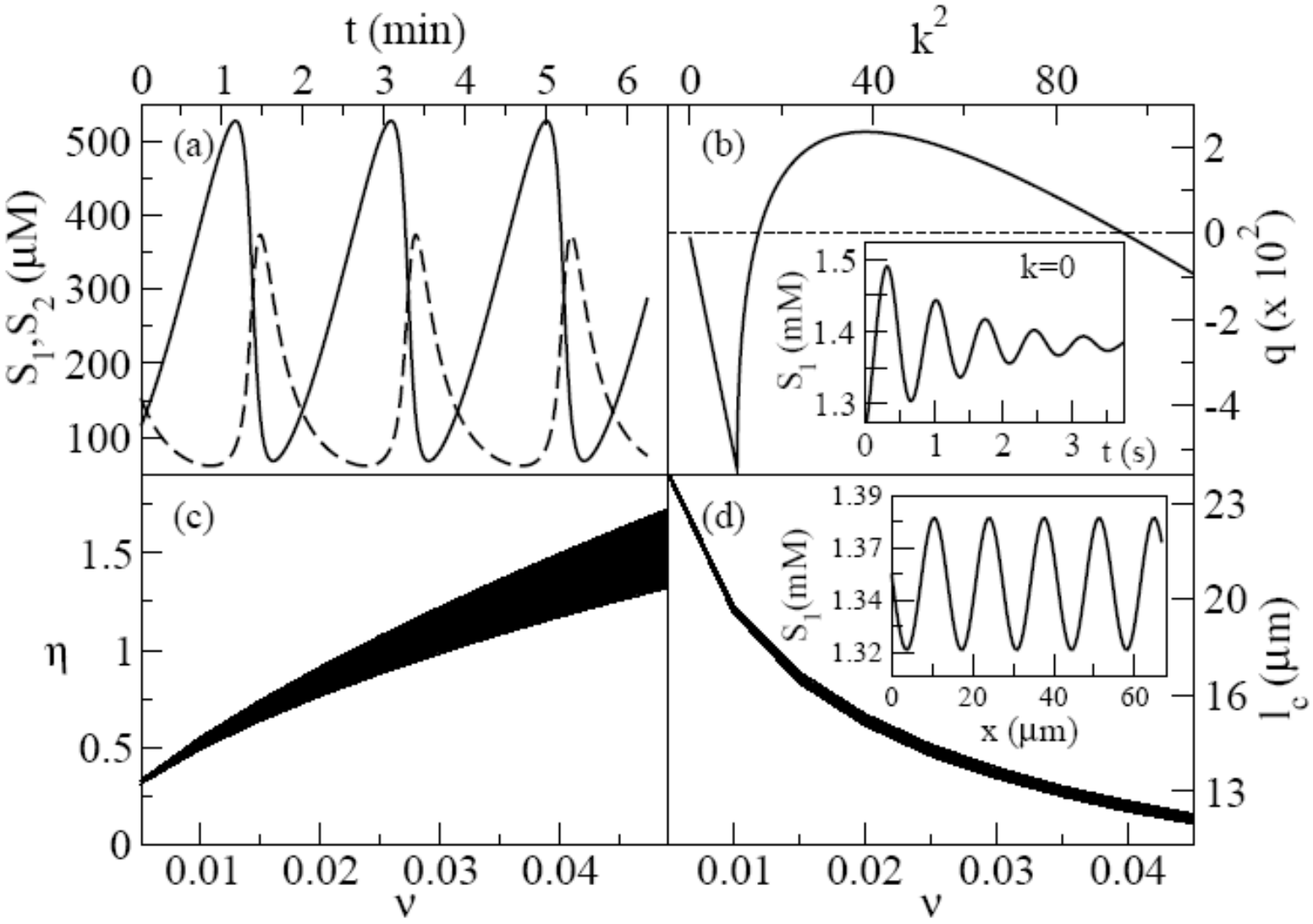
$$S_1ES_2^{\gamma} \xrightarrow{k_{+2}} ES_2^{\gamma} + S_2 \xrightarrow{\nu_2},$$

$$\gamma S_2 + E \underset{k_{-3}}{\overset{k_{+3}}{\leftrightarrow}} ES_2^{\gamma},$$

E=PFK; S_1=ATP (injected at rate ν_1);
S_2=ADP (removed at rate ν_2 *[ADP]*)



La activación de la PFK por el ADP provee el “feedback” positivo necesario para explicar las oscilaciones.



(a) Oscilaciones glicoliticas en ATP y ADP de acuerdo al modelo de Selkov

Modelo de Selkov.

Esquema de reacción

$$\xrightarrow{v_1} S_1,$$

$$S_1 + ES_2^{\gamma} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} S_1ES_2^{\gamma} \xrightarrow{k_2} ES_2^{\gamma} + S_2,$$

$$S_2 \xrightarrow{v_2}.$$

Con $s_1 = [S_1], s_2 = [S_2], e = [E], x_1 = [ES_2^{\gamma}], x_2 = [S_1ES_2^{\gamma}]$:

y $e + x_1 + x_2 = e_0$

las ecuaciones son:

$$\frac{ds_1}{dt} = v_1 - k_1 s_1 x_1 + k_{-1} x_2,$$

$$\frac{ds_2}{dt} = k_2 x_2 - \gamma k_3 s_2^{\gamma} e + \gamma k_{-3} x_1 - v_2 s_2,$$

$$\frac{dx_1}{dt} = -k_1 s_1 x_1 + (k_{-1} + k_2) x_2 + k_3 s_2^{\gamma} e - k_{-3} x_1,$$

$$\frac{dx_2}{dt} = k_1 s_1 x_1 - (k_{-1} + k_2) x_2.$$

Con cantidades adimensionales

$$\sigma_1 = \frac{k_1 s_1}{k_2 + k_{-1}} \quad \sigma_2 = \left(\frac{k_3}{k_{-3}}\right)^{1/\gamma} s_2, \qquad u_1 = x_1/e_0, u_2 = x_2/e_0, t = \frac{k_2 + k_{-1}}{e_0 k_1 k_2}\tau$$

Es

$$\frac{d\sigma_1}{d\tau} = v - \frac{k_2 + k_{-1}}{k_2} u_1 \sigma_1 + \frac{k_{-1}}{k_2} u_2,$$

$$\frac{d\sigma_2}{d\tau} = \alpha \left[u_2 - \frac{\gamma k_{-3}}{k_2} \sigma_2^{\gamma} (1 - u_1 - u_2) + \frac{\gamma k_{-3}}{k_2} u_1 \right] - \eta \sigma_2,$$

$$\epsilon \frac{du_1}{d\tau} = u_2 - \sigma_1 u_1 + \frac{k_{-3}}{k_2 + k_{-1}} \left[\sigma_2^{\gamma} (1 - u_1 - u_2) - u_1 \right],$$

$$\epsilon \frac{du_2}{d\tau} = \sigma_1 u_1 - u_2,$$

Donde

$$\epsilon = \frac{e_0 k_1 k_2}{(k_2 + k_{-1})^2}, v = \frac{v_1}{k_2 e_0}, \eta = \frac{v_2 (k_2 + k_{-1})}{k_1 k_2 e_0}, \alpha = \frac{k_2 + k_{-1}}{k_1} \left(\frac{k_3}{k_{-3}}\right)^{1/\gamma}$$

Ecuaciones (adimensionales)

$$\frac{d\sigma_1}{d\tau} = \nu - \frac{k_2 + k_{-1}}{k_2} u_1 \sigma_1 + \frac{k_{-1}}{k_2} u_2,$$

$$\frac{d\sigma_2}{d\tau} = \alpha \left[u_2 - \frac{\gamma k_{-3}}{k_2} \sigma_2^{\gamma} (1 - u_1 - u_2) + \frac{\gamma k_{-3}}{k_2} u_1 \right] - \eta \sigma_2,$$

$$\epsilon \frac{du_1}{d\tau} = u_2 - \sigma_1 u_1 + \frac{k_{-3}}{k_2 + k_{-1}} \left[\sigma_2^{\gamma} (1 - u_1 - u_2) - u_1 \right],$$

$$\epsilon \frac{du_2}{d\tau} = \sigma_1 u_1 - u_2,$$

Usando la aproximación cuasiestacionaria

$$u_1 = \frac{\sigma_2^{\gamma}}{\sigma_2^{\gamma} \sigma_1 + \sigma_2^{\gamma} + 1},$$

$$u_2 = \frac{\sigma_1 \sigma_2^{\gamma}}{\sigma_2^{\gamma} \sigma_1 + \sigma_2^{\gamma} + 1} = f(\sigma_1, \sigma_2),$$

resulta

$$\frac{d\sigma_1}{d\tau} = \nu - f(\sigma_1, \sigma_2),$$

$$\frac{d\sigma_2}{d\tau} = \alpha f(\sigma_1, \sigma_2) - \eta \sigma_2.$$

Y sus puntos fijos, satisfacen:

$$\sigma_1 = \frac{\nu}{1 - \nu} \frac{1 + \sigma_2^{\gamma}}{\sigma_2^{\gamma}} \qquad \left(\frac{d\sigma_1}{d\tau} = 0 \right),$$

(esto define las nulclinas)

$$\sigma_1 = \frac{1 + \sigma_2^{\gamma}}{\sigma_2^{\gamma - 1} (p - \sigma_2)} \qquad \left(\frac{d\sigma_2}{d\tau} = 0 \right),$$

$$\Rightarrow \quad \sigma_2 = p\nu,$$

$$\sigma_1 = \frac{\nu (1 + \sigma_2^{\gamma})}{(1 - \nu) \sigma_2^{\gamma}}.$$

con $p = \alpha / \eta$.

Ecuaciones linearizadas alrededor del pto fijo:

$$\frac{d\tilde{\sigma}_1}{d\tau} = -f_1 \tilde{\sigma}_1 - f_2 \tilde{\sigma}_2,$$

$$\frac{d\tilde{\sigma}_2}{d\tau} = \alpha f_1 \tilde{\sigma}_1 + (\alpha f_2 - \eta) \tilde{\sigma}_2,$$

con

$$f_j = \frac{\partial f}{\partial \sigma_j}, \; j = 1, 2$$

Simulación numérica: trayectoria + nulclinas

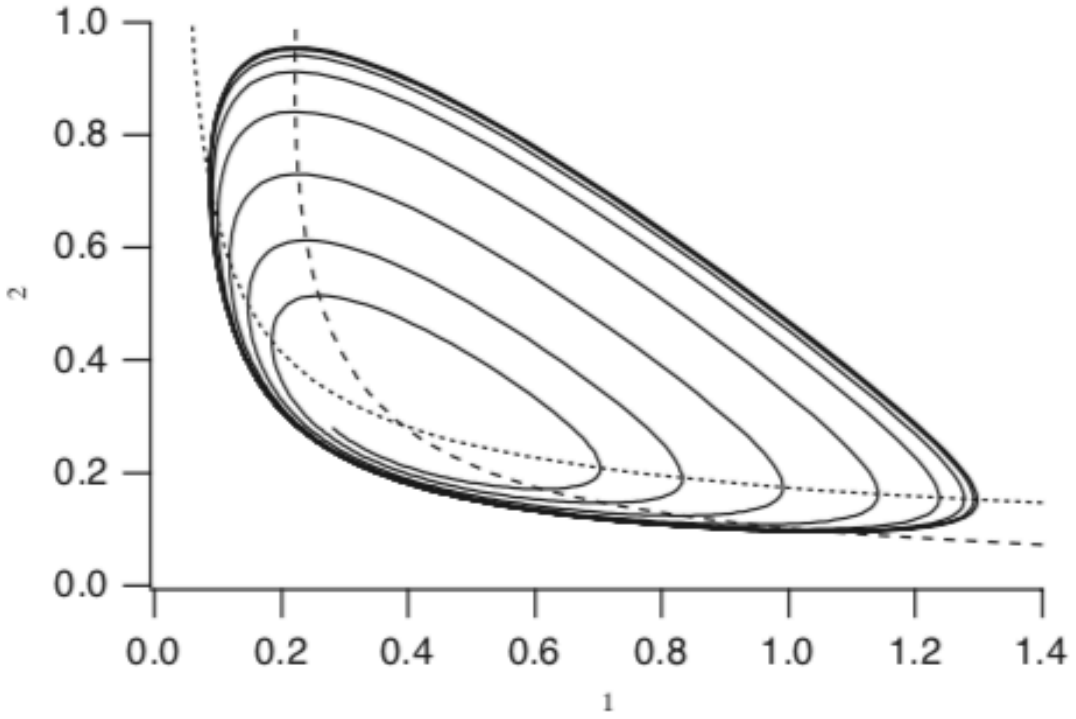


Figure 1.7 Phase portrait of the Sel'kov glycolysis model with $\nu = 0.0285, \eta = 0.1, \alpha = 1.0,$ and $\gamma = 2$. Dotted curve: $\frac{d\sigma_1}{d\tau} = 0$. Dashed curve: $\frac{d\sigma_2}{d\tau} = 0$.