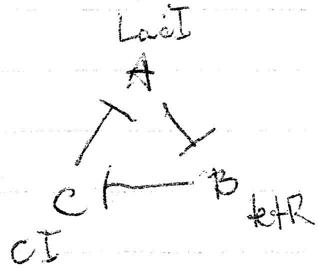
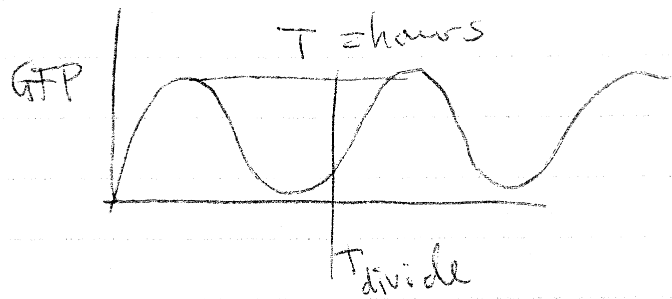
Oscillations & Poincaré Thm

Repressilator Elowitz & Leibler: Nature 403, 335 (2000)





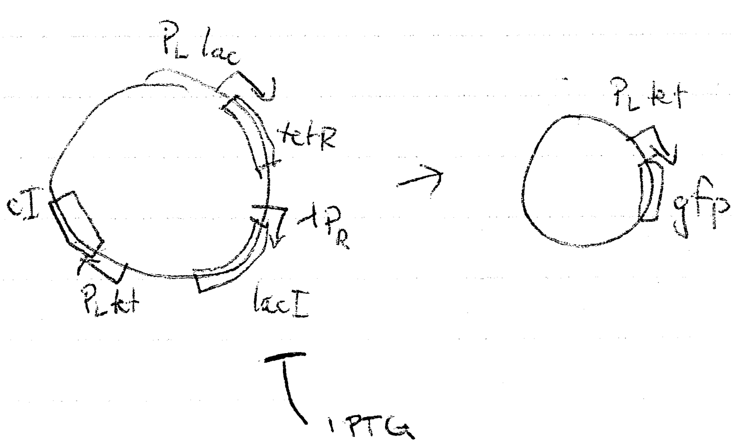
Solution depends on: transcription rate, translation rate, decay rates

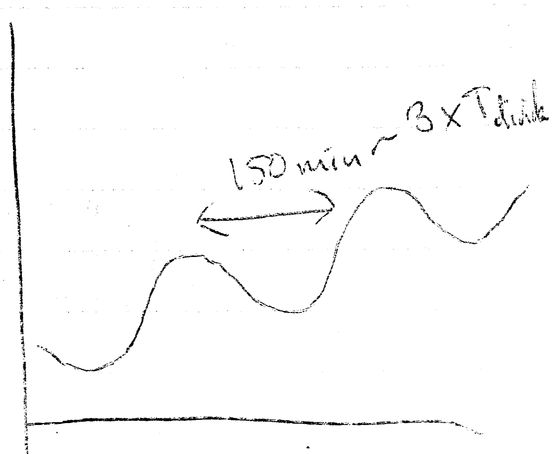
→ 1 stable state, or sustainable limit cycle

Oscillations: strong promoter, good RBS, good repression, cooperativity, comparable decay rates

mRNA decay rates ~ 2 min in E. coli

protein rates ~ 60 min → 4 min @ protease tag





- repressilator stopped in stationary phase
∴ coupled to global cell growth

<u>Theory:</u>

leakiness

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_j^n)} + \alpha_0$$

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

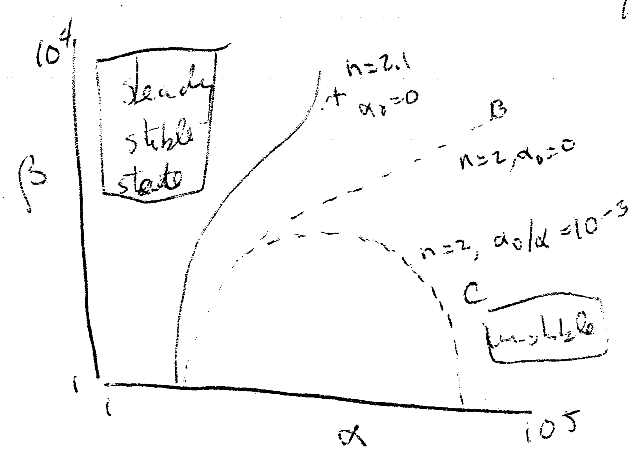
chose: $n=2$, promoter strength $\approx 5\times10^{-4} \to 0.5$, avg trans. eff = 20 ~~tran~~ proteins/transcript, protein half life = 10 min
mRNA half life = 2 min, K_m = # of rep for ½ max = 40

stable → unstable when $\frac{(\beta+1)^2}{\beta} < \frac{3X^2}{4+2X}$

where

$$X = \frac{\alpha n p^{n-1}}{(1+p^n)^2}$$

p is solution to, $p = \frac{\alpha}{1+p^n} + \alpha_0$



+ve feedback (15)

Oscillators – 2 slow variables + delay

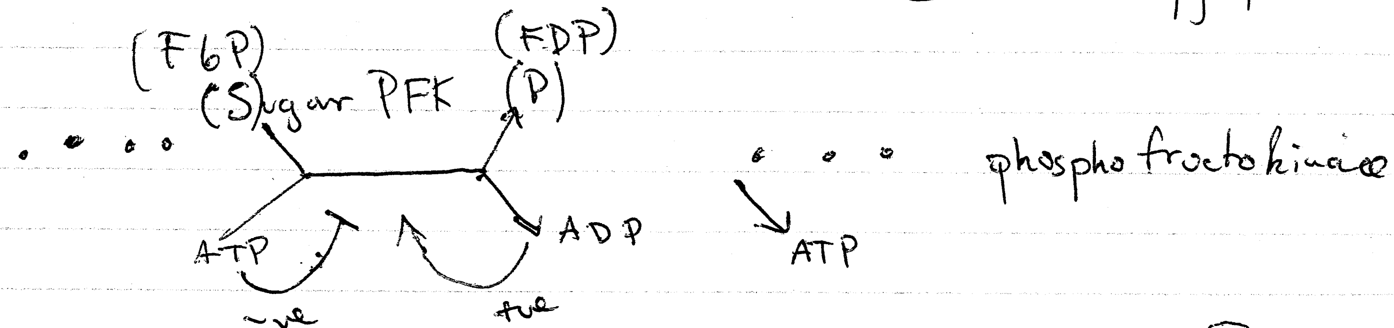
- Lots of bio oscillators!

  - cell cycle, circadian rythms, metabolic oscillations, heart beat.

- Glycolytic oscillations (Metabolism) (sustained oscil 1969) (CREBS)

Remember: glucose → (enzymes) → 2ATP + pyruvate

↑ energy production



[SHOW FIGURES]

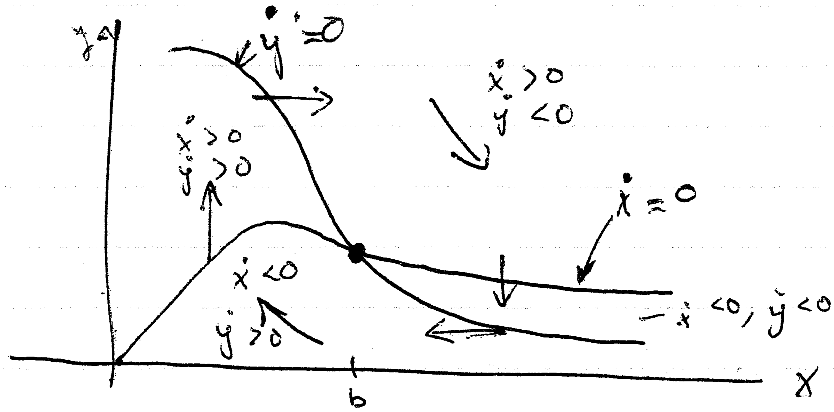
allosteric enzyme = binds ligand which makes it activity change

2 equns @ +ve & –ve feedback (key)

$$\begin{aligned} (P) \quad \dot{x} &= -x + \overset{(-ve)}{a y} + \overset{(+ve)}{x^2 y} \\ (S) \quad \dot{y} &= \underset{\text{source}}{b} - a y - x^2 y \end{aligned}$$

(Goldbeter & Lefever 1972)

argue $P\uparrow$ $S\downarrow$ as $P\uparrow$ $\therefore$ $P\downarrow$ & $S\uparrow$

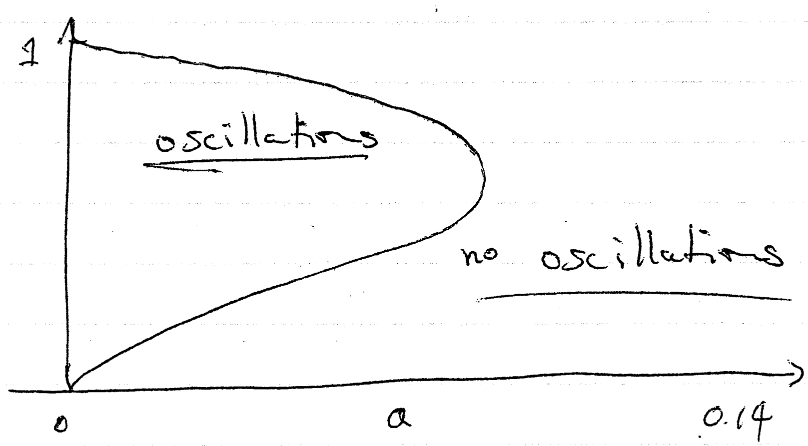


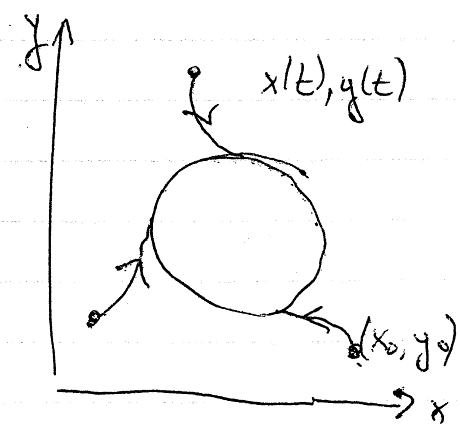
phase plane

fixed point:
$$y = \frac{x}{a+x^2} \quad \& \quad y = \frac{b}{a+x^2}$$

$$\Rightarrow \quad x^* = b \quad \& \quad y^* = \frac{b}{a+b^2}$$

(Finish) Want to Show:





Nullclines:

$$y = \frac{x}{a+x^2} \quad ; \quad y = \frac{b}{a+x^2}$$

$\Rightarrow$ ONE fixed pt: $x^* = b$ & $y^* = \dfrac{b}{a+b^2}$.

Stability:

$$\dot{x} = f(x,y) = -x + ay + x^2 y$$

$$\dot{y} = g(x,y) = b - ay - x^2 y$$

$$A = \begin{pmatrix} \partial f/\partial x & \partial f/\partial y \\ \partial g/\partial x & \partial g/\partial y \end{pmatrix} = \begin{pmatrix} -1 + 2x^* y^* & a + (x^*)^2 \\ -2xy & -(a + (x^*)^2) \end{pmatrix}$$

Stability: need $\Delta = \det(A) > 0$

$\tau = \mathrm{Tr}(A) < 0$

so $\tau = -(a + b^2 + 1) + \dfrac{2b^2}{b^2 + a}$

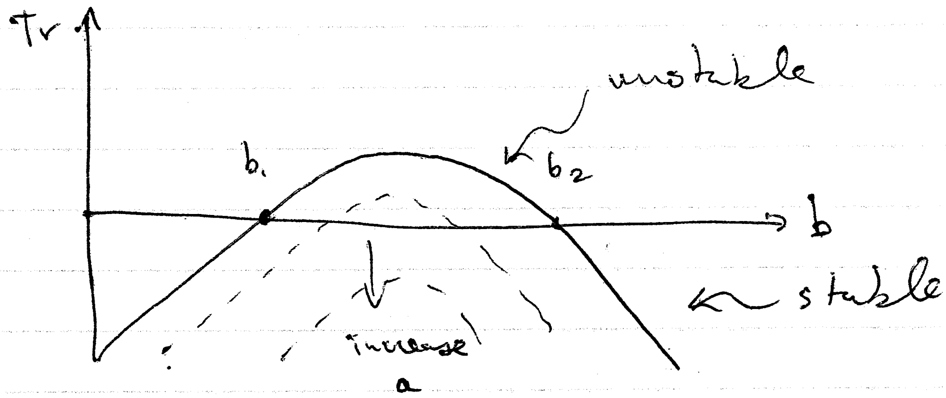
and

$$\Delta = a + b^2$$

Limits: $\tau(b=0) = -(a+1) < 0$!!!

$\tau(b=\infty) = -(b^2+a+1)+2 < 0$!!!

$\therefore$ small b & large b $\rightarrow$ stable fixed point



for $b_1 < b < b_2$

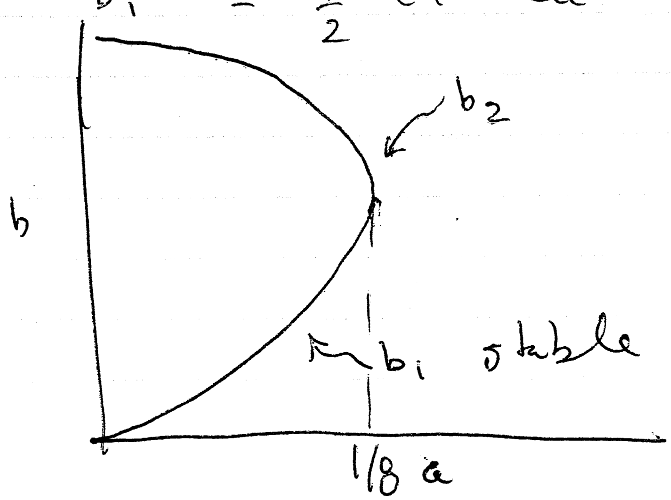
$\rightarrow$ unstable

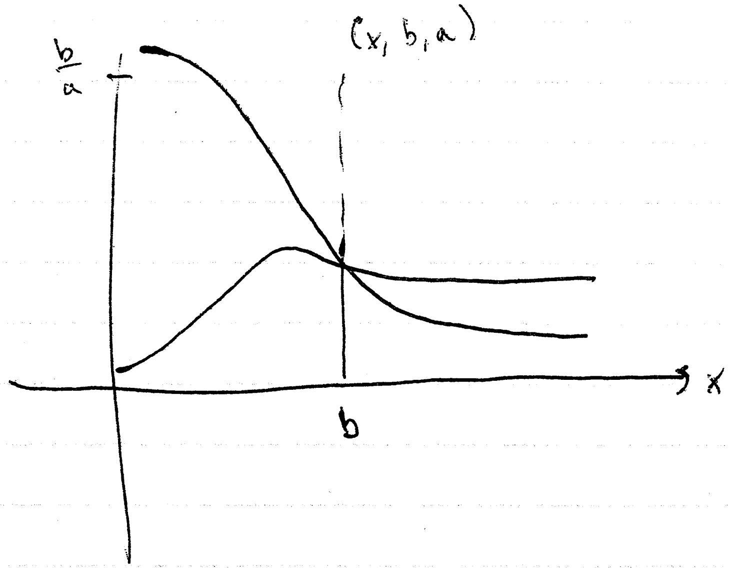
Cross-over: $Tr = 0$

$$\Rightarrow \quad \frac{2b^2}{b^2+a} = a + b^2 + 1$$

$$\rightarrow \quad b_2^2 = \frac{1}{2}\left(1 - 2a + \sqrt{1-8a}\right)$$

$$b_1^2 = \frac{1}{2}\left(1 - 2a - \sqrt{1-8a}\right)$$





eigenvalues:

$$\lambda_{1,2} = \frac{Tr \pm \sqrt{\tau^2 - 4\Delta}}{2}$$

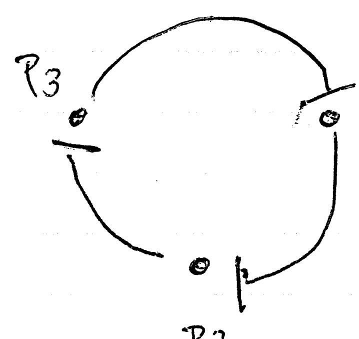
@ bifurcation $\lambda_{1,2} = \pm i\omega$; $\tau = 0$

where

$$\omega = \sqrt{\Delta} = \sqrt{a^2+b^2}$$

Show limit cycle!?!

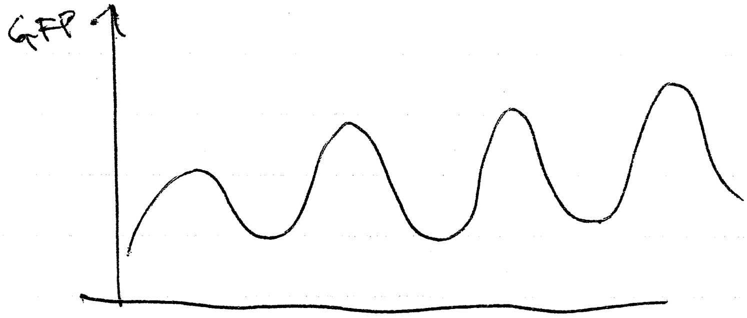
Synthetic Oscillators — Elowitz & Leibler (Repressilator)



$$\frac{dp_i}{dt} = -p_1 + \frac{\alpha}{1+p_3^n} + \alpha_0$$

$$\frac{dp_1}{dt} = -p_2 + \frac{\alpha}{1+p_1^n} + \alpha_0$$

$$\frac{dp_3}{dt} = -p_3 + \frac{\alpha}{1+p_2^n} + \alpha_0$$



Now symmetry: $p \equiv p_1 = p_2 = p_3$

so @ steady-state

$$p = \frac{\alpha}{1+p^n} + \alpha_0$$

Jacobian:

$$A = \begin{bmatrix} -1 & 0 & X \\ X & -1 & 0 \\ 0 & X & -1 \end{bmatrix}$$

where

$$X = -\frac{\alpha n p^{n-1}}{(1+p^n)^2} \qquad (<0)$$

evalues: $-(1+\lambda)^3 + X^3 = 0$

$\lambda_1 = X - 1$ (decays)

$\lambda_2 = -1 - \frac{1}{2}X + i\frac{\sqrt{3}}{2}X$ (could oscillate)

$\lambda_3 = -1 - \frac{1}{2}X - i\frac{\sqrt{3}}{2}X$

Need all $Re(\lambda) < 0$ for stability

$X < 0$

so

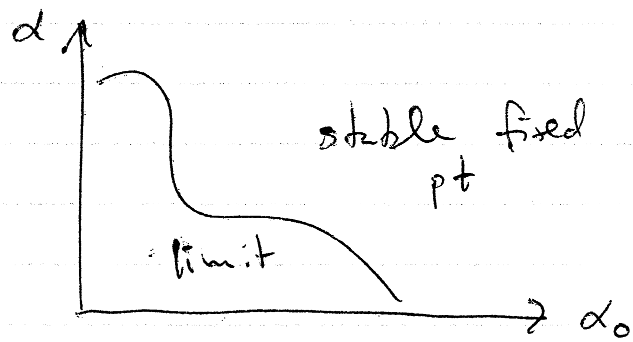
$-1 - \frac{1}{2} X < 0 \quad \rightarrow \quad X > -2$

so

$-2 < X < 0$

or $\frac{\alpha n p^{n-1}}{(1+p^n)^2} < 2 \quad = 2$ @ boundary

gives $\alpha = f(\alpha_0)$ & $p = g(\alpha, \alpha_0)$

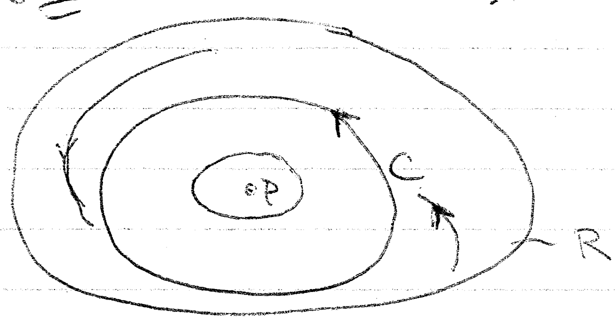


Existence of Limit Cycles

- Want to show the existence of an attracting stable closed orbit
- Theorems for systems that cannot have limit cycles:
  - a) 1D systems
  - b) $\dot{x} = -\nabla V(x)$ ← no oscillation

Poincaré - Bendixson Theorem (Existence of cycle) (2D)!!!

(no chaos in 2D!!!)



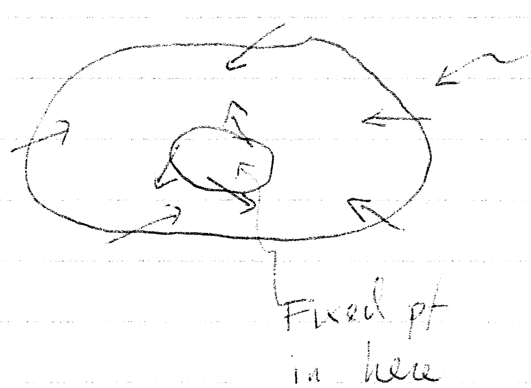
a) R is closed bounded set

b) $\dot{\vec{x}} = f(\vec{x})$ is differentiable vector field on R

c) No fixed points IN R

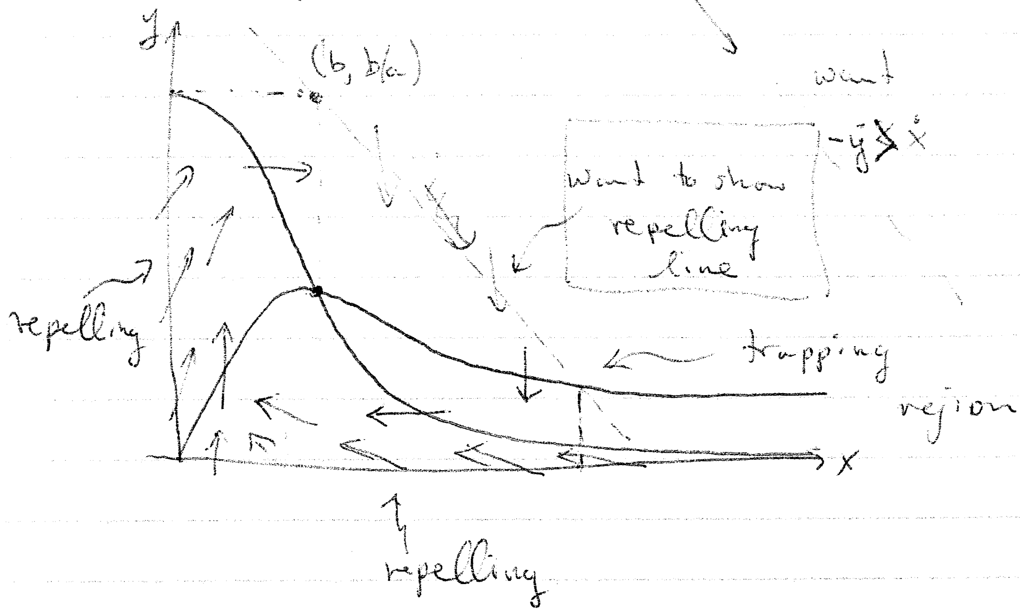
→ Then there is a "closed" orbit

Physicist Intuitive Proof:



if boundaries are repelling then there will be a closed orbit.

Back to Glycolysis:



$$\dot{x} = -x + ay + x^2 y$$

$$\dot{y} = b - ay - x^2 y$$

slope

@ large x: $\quad \dot{x} \approx x^2 y \quad$ & $\dot{y} \approx -x^2 y \quad$ so $\dfrac{\dot{y}}{\dot{x}} = \dfrac{dy}{dx} = -1$

- compare $\dot{x}$ with $|\dot{y}|$:

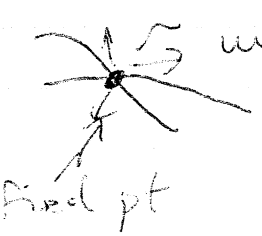
$$-\dot{y} - \dot{x} = -b + \cancel{ay} + \cancel{x^2 y} + x - \cancel{ay} - \cancel{x^2 y}$$

$$= x - b > 0 \quad \text{for } x > b$$

so $-\dot{y} > \dot{x}$ for $x > b$! ~~hence~~

$\Rightarrow$ can draw a line from $x = b$ with slope of -1 & know its repelling!

- Need interior boundary that is also repelling



unstable & repellant (did this last class!)

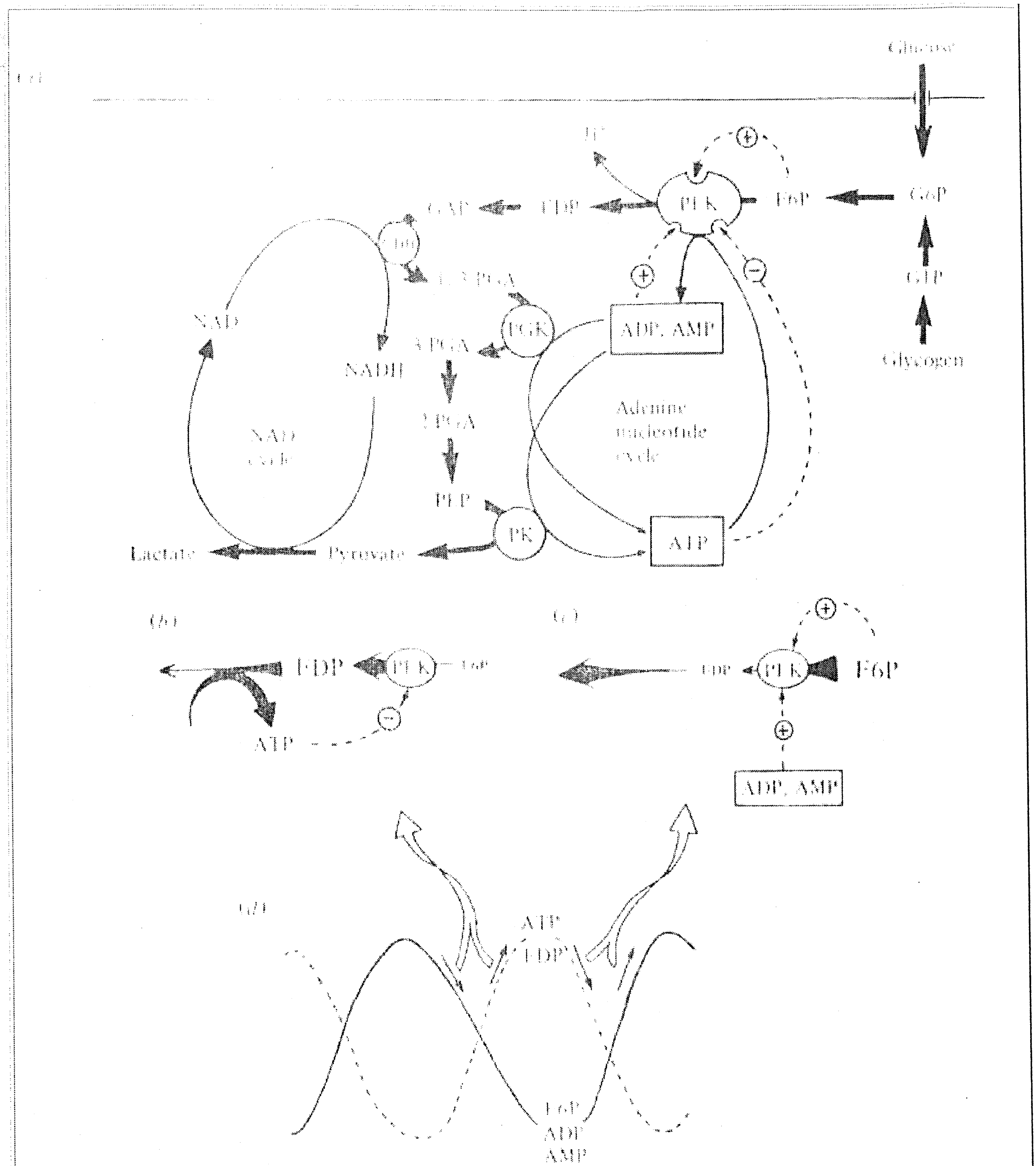


Fig. 3. The glycolytic oscillator. (a) A summary of the glycolytic pathway (thick arrows) together with the NAD and adenine nucleotide cycles (thin arrows). The dotted lines represent the allosteric control of phosphofructokinase (PFK) by ATP, ADP, AMP and fructose-6-phosphate (F6P). The circles indicate those enzymes which seem to be important for oscillatory activity: GDH-glyceraldehyde dehydrogenase; PGK-phosphoglycerate kinase; PK-pyruvate kinase. (b) and (c) Changes in the concentration of key intermediates at two points during the oscillatory cycle. Large-face lettering of metabolites is used to indicate high

Control of Oscillations by Substrate Input.

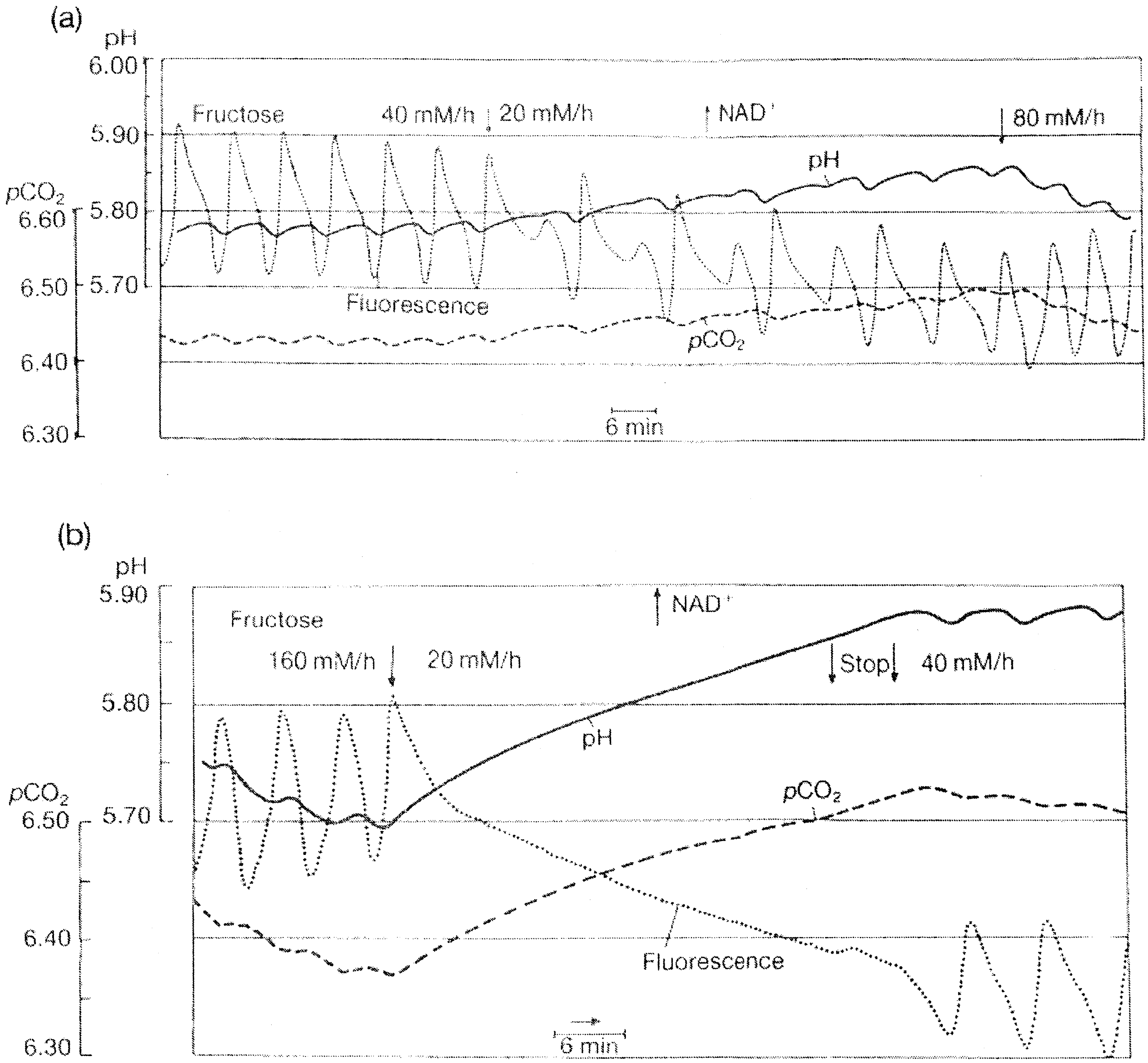


Fig. 2.4. Control of glycolytic oscillations in yeast extracts by the substrate injection rate. (a) The diminution of the rate of injection of fructose from 40 to 20 mM/h causes a lengthening of the period as well as a change in the waveform of oscillations; this change is reversible. (b) Decreasing the injection rate below 20 mM/h causes the reversible suppression of the oscillations (Hess & Boiteux, 1968b).

[Reproduced from Goldbeter (1996).]