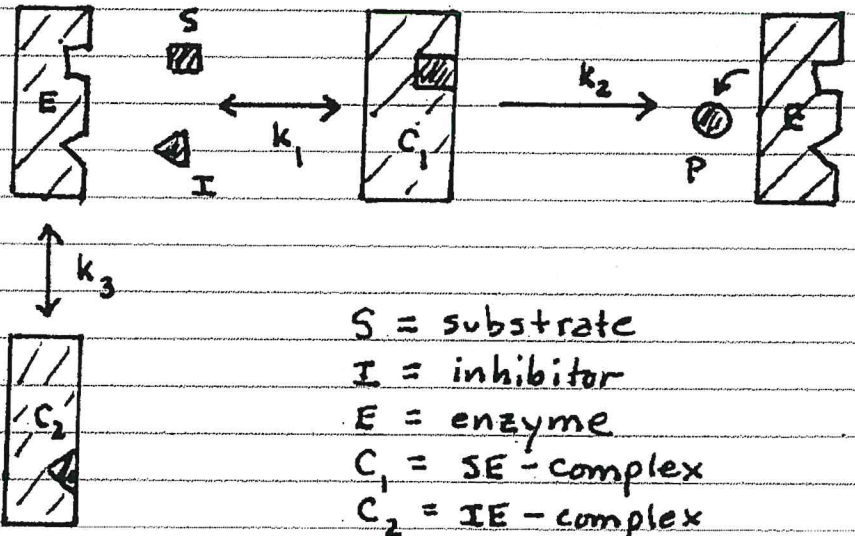
Competetive Inhibition



S = substrate
I = inhibitor
E = enzyme
C_1 = SE - complex
C_2 = IE - complex
P = product

Enzyme has two binding sites but only SE complex can make product P so I acts to inhibit such production.

Law of mass action ($\varepsilon = E_0/S_0 \ll 1$ assumed)

(1) $S' = -k_1 SE + k_{-1} C_1$ } slow

(2) $I' = -k_3 IE + k_{-3} C_2$ } slow

(3) $C_1' = k_1 SE - (k_{-1} + k_2) C_1$ } fast

(4) $C_2' = k_3 IE - k_{-3} C_2$ } fast

(5) $E' = -C_1' - C_2'$ conserved receptors

(6) $P' = k_2 C_1$ production rate

Reaction Equations

$$S + E \underset{}{\overset{k_1}{\longleftrightarrow}} C_1 \xrightarrow{k_2} E + P$$

$$I + E \overset{k_3}{\longleftrightarrow} C_2$$

With nondimensionalizing we know (3)-(4) will be fast. If E, C_K are scaled by E_0 and S by S_0 the dimensionless forms will be

$$\varepsilon \dot{C}_K = O(1) \qquad \varepsilon = \frac{E_0}{S_0} \ll 1$$

Quasi Steady State

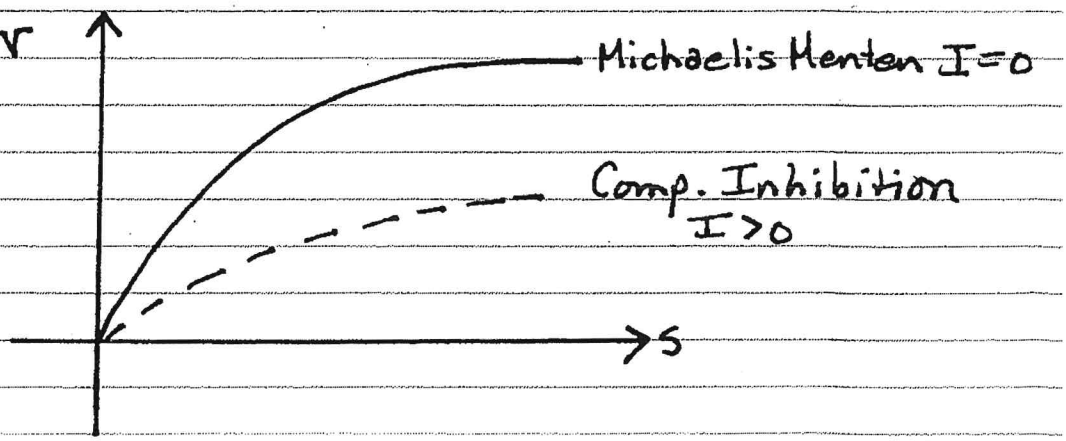
Set $C_1' = C_2' = 0$ and use conservation law (5) to solve for C_1 in terms of slow variables S, I to ultimated get production rate

$$\frac{dP}{dt} = V = \frac{\bar{V} S}{S + K_m (1 + I/K_i)}$$

↑ inhibition

where $\bar{V} = k_2 E_0$ maximal rate and

$$K_m = \frac{k_{-1} + k_2}{k_1} \qquad K_i = \frac{k_{-3}}{k_3}$$

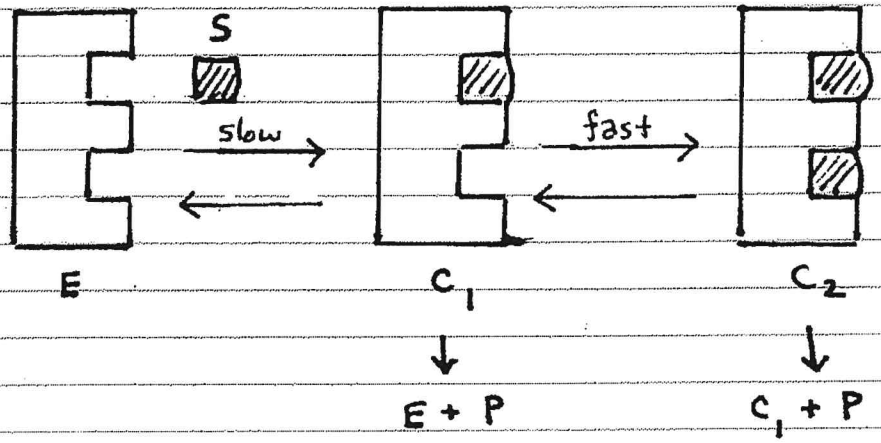


Cooperativity

$$S + E \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} C_1 \xrightarrow{k_2} E + P$$

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} C_2 \xrightarrow{k_4} C_1 + P$$

Cartoon



Indicated rates k_1 and k_3 are, respectively, assumed to be small and large. System favors cooperative C_2 state.

QSS Approximation, conservation receptors, $\varepsilon = \frac{E_0}{S_0} \ll 1$

$$\bar{V} = \frac{dP}{dt} = \frac{(k_2 K_2 + k_4 S') E_0 S'}{K_1 K_2 + K_2 S' + S'^2}$$

where

$$K_1 = \frac{k_{-1} + k_2}{k_1} \qquad K_2 = \frac{k_4 + k_{-3}}{k_3}$$

Large Positive Cooperativity

As indicated we assume (see cartoon) that k_1 is relatively small while k_3 is relatively large:

$$k_1 = \delta \ll 1 \qquad k_3 = \frac{k}{\delta} \gg 1$$

for some constant k. Then V is a function of S

$$V = V(S) \qquad S \ll 1$$

Since $V \sim V(0)$ we have an approximate production rate

$$(1) \qquad V = \frac{\bar{V} S^2}{K + S^2} \qquad K = K_1 K_2$$

where $\bar{V} = k_4 E_0$. $V(S)$ is a Hille function of Hille coefficient $n = 2$.

n-site Positive Cooperativity

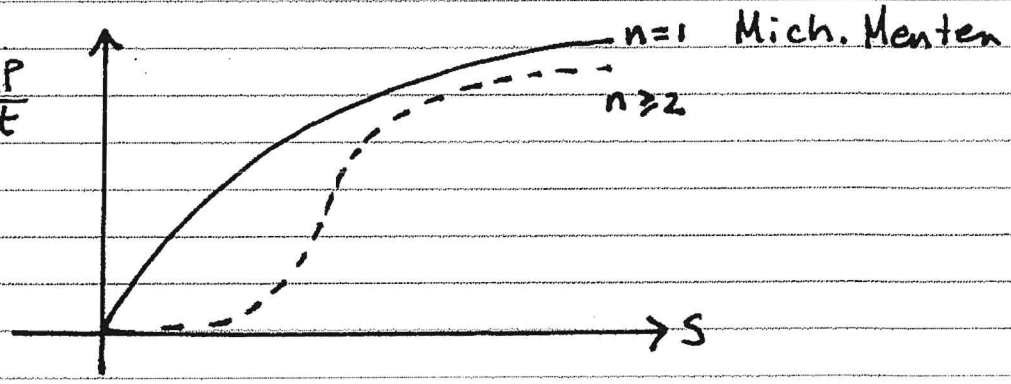
Assume formation of C_1 is slow ($k_1 \ll 1$) but formation C_k, $k \geq 1$ fast.

Here E has n-sites and C_k = complex of E with k-substrate molecules attached.

Same analysis

$$\frac{dP}{dt} = V = \frac{\bar{V} S^n}{K + S^n}$$ Hill eqn, coeff n

where $K = \prod_{j=1}^{n} K_j$



Experimental Correlation

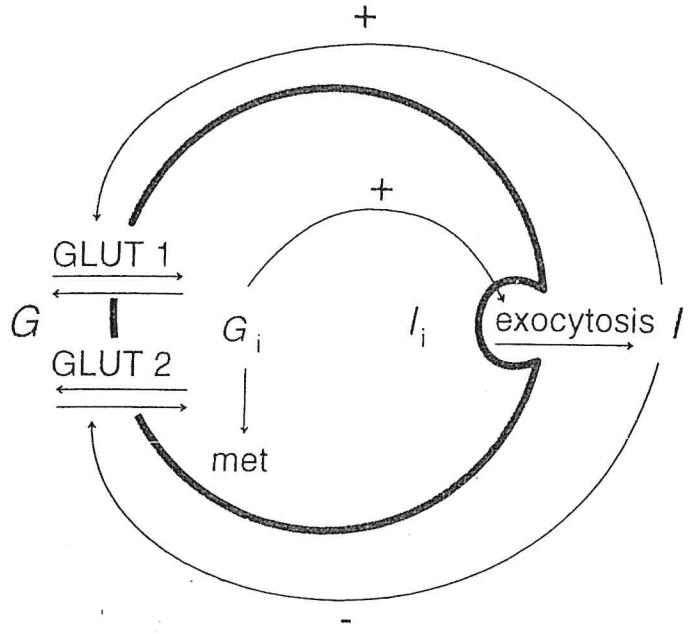
$$n \ln S = n \ln K + \ln\left(\frac{V}{\bar{V} - V}\right)$$

$$n \alpha_k = n \ln K + \beta_k$$

Data $\{(\alpha_k, \beta_k)\}$ and linear regression for n, K.

Glucose-dependent insulin secretion

Pancreatic β-cells secrete insulin I via exocytosis at a rate dependent on intra and extracellular glucose.



The following rates and assumptions apply

R_1 = rate of transport into cell through insuline <u>activated</u> GLUT 1 transporters

R_2 = rate of transport into cell through insulin inactived GLUT 2 transporters

R_s = insulin production rate

R_m = intracellular glucose metabolism rate

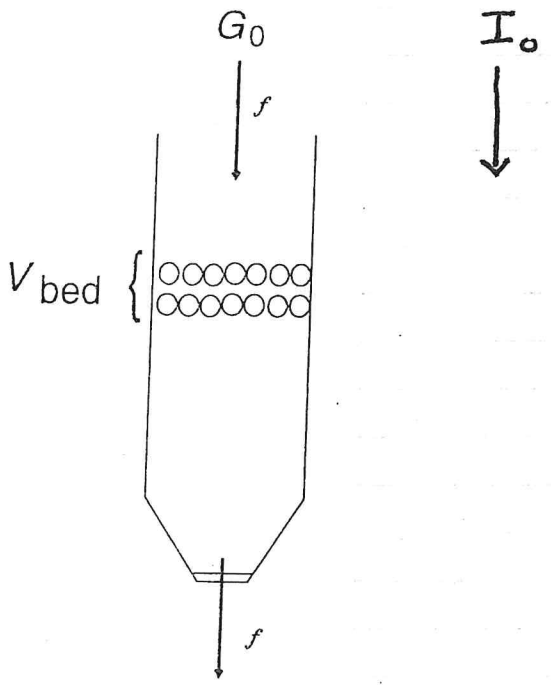
G = extracellular glucose

G_i = intracellular glucose

I = insulin

J = inhibition variable

Experimentally β-cells in a bed (V_{bed}) and glucose of concentration G_0 is continuously flushed through at flow rate f:



Controlled: inflow G_0, I_0

measured: outflow G, I

Nondimensionalized model (Fall et al, pg 87)

(1) $g' = -R_1 - R_2 - k_0 (g - g_0)$

(2) $g_i' = R_1 + R_2 - R_m$

(3) $i' = R_s - k_0 i$

(4) $j' = j_\infty(i) - j$ (phenomenological)

where

$$R_1 = \frac{K_1 V_1 (g - g_i)}{(K_1 + g)(K_1 + g_i)} \cdot \frac{i^n}{K_i^n + i^n} \quad \oplus$$

$$R_2 = \frac{K_2 V_2 (g j^m - g_i)}{(K_2 + g)(K_2 + g_i)} \quad \ominus$$

$$R_m = \frac{V_m g_i}{1 + g_i}$$ Mich-Menten QSS

$$R_s = \frac{V_s (R_m^4 + L^4)}{K_s^4 + R_m^4 + L^4}$$ (phenomenological)

The latter assumes insulin secretion rate that grows with glucose metabolism rate. Looks like 4-site cooperativity (were it not for L). Lastly

$$j_\infty(i) = \frac{1}{1 + i}$$

Model Parameters

Table 4.1 Standard Dimensional Parameters

Fixed by experiment	V_m	0.24 mM/min
	K_m	9.8 mM
	V_s	0.034 mM/min
	K_s	0.13 mM/min
	V_{max2}	32.0 mM/min
	K_2	17.0 mM
	V_{max1}	120.0 mM/min
	K_1	1.4 mM
	L	0.01 mM/min
Experimentally variable	k_0	400.0/min
	I_0	0.0 mM
	G_0	8-22 mM
Adjustable in the model	K_I	1×10^{-6} mM
	K_i	4.0×10^{-5} mM
	τ	20.0 min

Table 4.2 Standard Dimensionless Parameters

Dimensionless parameter	Dimensional definition	Standard value	
$\hat{V}_m$	$\tau V_m/K_m$	0.50	
$\hat{V}_s$	$\tau V_s/K_I$	6.8×10^5	large
$\hat{K}_s$	$\tau K_s/K_m$	0.27	
$\hat{V}_{max2}$	$\tau V_{max2}/K_m$	65.3	
$\hat{K}_2$	K_2/K_m	1.7	
$\hat{V}_{max1}$	$\tau V_{max1}/K_m$	245.0	
$\hat{K}_2$	K_1/K_m	0.14	
$\hat{L}$	$\tau L/K_m$	0.02	
$\hat{k}_0$	τk_0	8×10^3	large
$\hat{G}_0$	G_0/K_m	0.8-2.2	
$\hat{K}_i$	K_i/K_I	40.0	

Fast-slow dynamics

Since $k_0 \gg 1$ in

$$g' = -R_1 + R_2 - k_0 (g - g_0)$$
$$i' = R_S - k_0 i$$

yields (QSS) approximations:

$$0 = -R_1 + R_2 - k_0 (g - g_0)$$
$$0 = R_S - k_0 i$$

Using these one can eliminate (g, i) to get a slow subsystem of the form

$$g_i' = F_1(g_i, j)$$
$$j' = F_2(g_i, j)$$

This system has oscillations!

Passive Transport

Transport of S from high to low concentrations through a membrane transporter such as extracellular glucose into cells. No energy input is required.

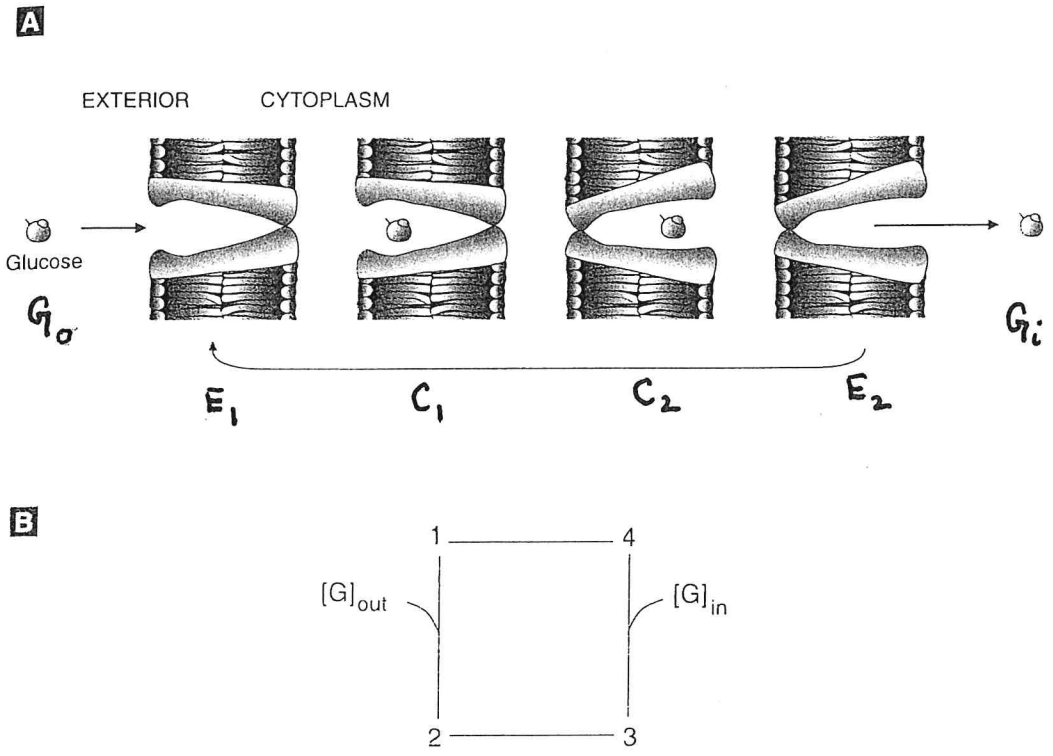


Figure 3.1 (A) Cartoon of four states of a GLUT transporter, showing the empty pore facing the exterior of the cell, glucose bound facing the exterior, glucose bound facing the interior, and the open pore facing the interior of the cell. Adapted from Leinhard et al. (1992). (B) Four-state kinetic diagram of a GLUT transporter based on the cartoon in (A).

E_1 = one unbound conformational state
C_1 = one bound conformational state
C_2 = another bound conformational state
E_2 = another unbound conformational state

(QSS)

$$V = \frac{dG_i}{dt} = \frac{\alpha_0 + \alpha_1 G_o + \alpha_2 G_i + \alpha_3 G_o G_i}{\beta_0 + \beta_1 G_o + \beta_2 G_i + \beta_3 G_o G_i}$$

SERCA Pumps $[Ca^{2+}]_o > [Ca^{2+}]_i$

pump Ca^{2+} up gradient

Rate of Ca^{2+} accumulation inside cell measured by rate of radioactive $^{45}Ca^{2+}$ uptake at ER (endoplasmic reticullum)
Conversion of ATP to ADP generates energy (loss of PO^4 phosphate group)

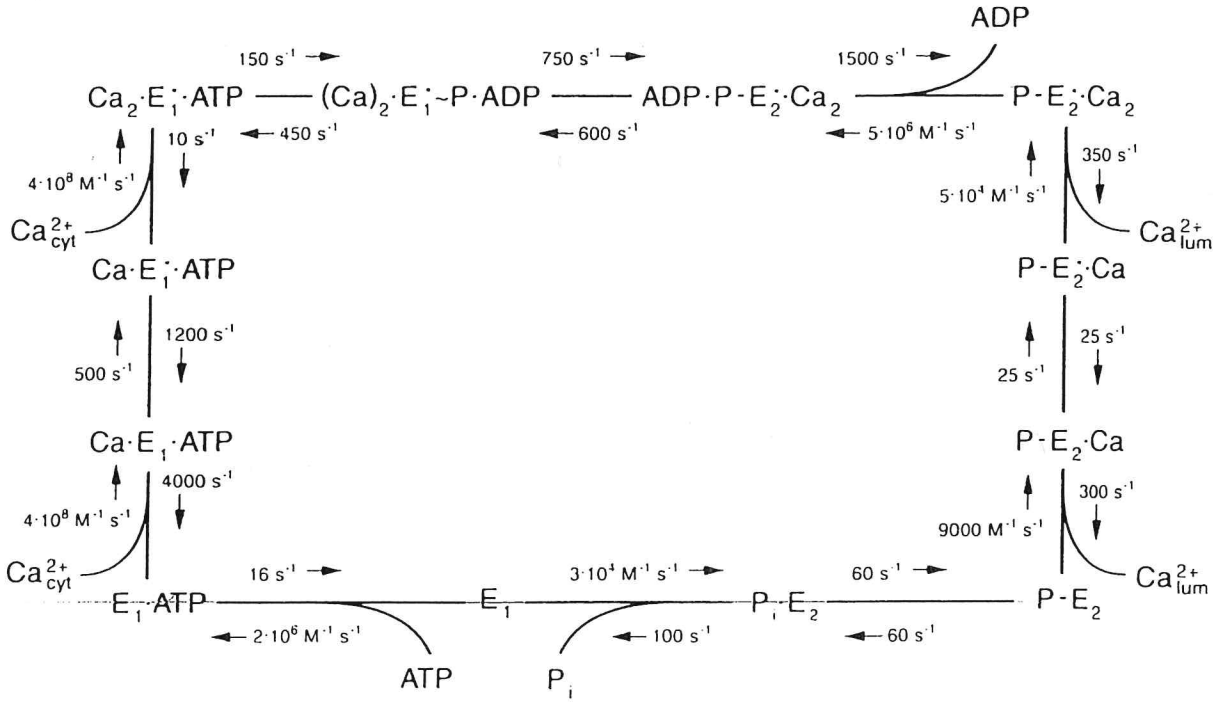


Figure 3.14 A twelve–state model of the SERCA pump. Note the two sequential Ca^{2+} binding steps on the left–hand side. Although the cycle is driven by the hydrolysis of ATP, all of the steps in the diagram contribute to the steady–state rate. Redrawn from Läuger (1991).

Twelve State model well approximated by 2-site cooperativity model hence intracellular production rate (QSS)

$$V = \frac{dCa_i}{dt} = \frac{\bar{V}\,[Ca_i]^2}{\bar{K}^2 + [Ca_i]^2}$$

Chemical Oscillators - Brusselator (1968)

Mostly inorganic chemical species.

$A = [BrO_3^-]$

$B =$ organic species

$X = [HBrO_2]$

$Y = [Br^-]$

where the reactions are

$$A \xrightarrow{k_1} X$$

$$B + X \xrightarrow{k_2} Y + D$$

$$2X + Y \xrightarrow{k_3} 3X$$

$$X \xrightarrow{k_4} E$$

Here D and E are other products.

It is assumed that the concentrations of A, B are artificially maintained at (nearly) constant levels. Resulting nondimensimalized eqns are called the Brusselator

Autocatalysis since X involved in its own production.

$$(1) \quad \dot{x} = a - (b+1)x + x^2 y$$

$$(2) \quad \dot{y} = bx - x^2 y$$

where (x, y) are dimensionless (I, I).

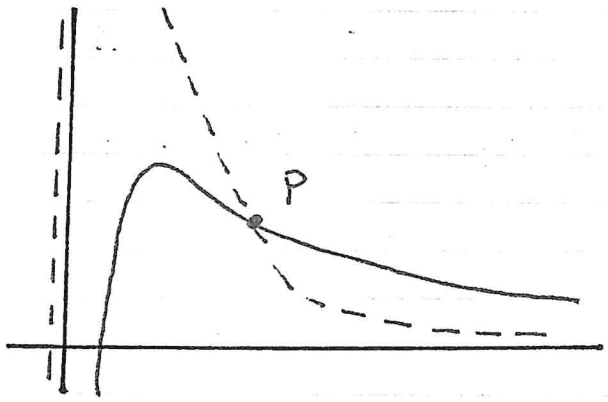
Null clines, equilibria

$\dot{x} = 0$ $\qquad y = F(x) = \frac{(b+1)}{x} - \frac{a}{x^2}$

$\dot{y} = 0$ $\qquad x = 0, \; y = \frac{b}{x}$

Sole equilibria

$$P = \left(a, \frac{b}{a}\right)$$



- - - - - $\dot{y} = 0$

——— $\dot{x} = 0$

Jacobian at P

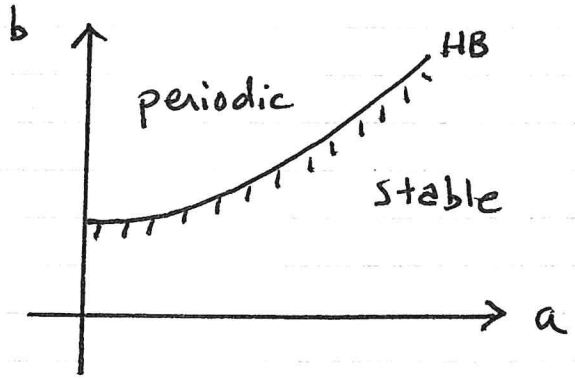
$$DF(P) = \begin{bmatrix} b-1 & a^2 \\ -b & -a^2 \end{bmatrix}$$

from which

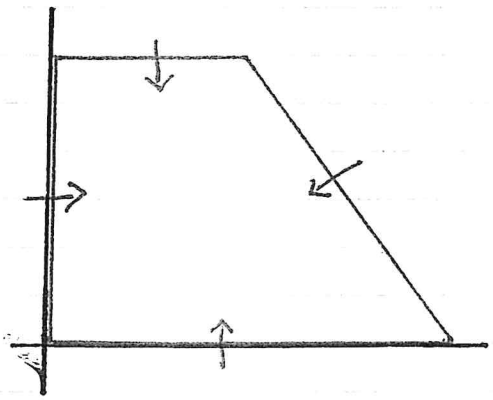
$$\det DF(P) = a^2 > 0$$

$$\text{Tr } DF(P) = b - 1 - a^2$$

Hopf bifurcations occur when Tr DF(P)



Hopf points alone only prove small amplitude periodic orbits



Trapping region shape to prove existence given Tr DF(P) > 0