Chapter 15

Teaching Reaction Equilibrium Using *Stella* Modeling Software

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Introduction

Equilibrium of biochemical reactions is seldom taught in labs of introductory biology courses. However, equilibrium is an excellent topic for the introduction of mathematical modeling into the lab curriculum.   First, a two-substrate reaction equilibrium is probably the simplest system that can be realistically modeled, so it is a natural application for mathematics in biology.   Second, equilibrium is a very abstract topic, but modeling can turn it into a hands-on exploration.   *Stella* modeling software facilitates the exercise by allowing students to construct sound models without common mathematical errors.

The attached student exercise is used in introductory biology laboratories at Clemson.   It asks students to model one of the reactions of glycolysis, the isomerization of glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP).   Because there is only one substrate on each side of this reaction, it is exceptionally easy to model.

Materials

Aside from either Windows or Macintosh computers sufficient to provide one computer for every 2-3 students, the exercise requires *Stella* Version 6.0 or 7.0. *Stella* can be obtained from:

> High Performance Systems, Inc.
> 45 Lyme Road, Suite 300
> Hanover, NH 03755
> 1-800-332-1202 (voice)
> (603) 643-9402 (fax)
> http://www.hps-inc.com

Stella is sold in "Basic" and "Research" versions; for the exercise shown here, the Basic version will suffice. Differences between the Basic and Research versions are discussed in Appendix D. HPS sells multiple copies of Stella in units called Lab Packs. In spring of 2001, Basic version prices for a Lab Pack of 5-14 units was $100 US

per unit, for 15-19 units it was $80 US per unit, and for 20-24 units it was $70 US/unit.   Single-unit prices are $299 US for the Basic version and $549 US for the Research version.

Notes for the Instructor

This exercise was performed as part of a larger lab on cellular respiration. Students can complete it in less than an hour with little trouble, although achieving understanding of the underlying principles of thermodynamics is a more elusive goal. At the time the students perform this exercise, they have had a previous introduction in lecture to reaction equilibrium and to elementary thermodynamics. The students were also familiar with the basics of glycolysis when they did the exercise.

The student exercise uses two *Stella* models. Exercise 1 uses a "bare-bones" model of a simple equilibrium reaction in which the students vary temperature and concentration of reactants. This is constructed by the students using the step-by-step instructions in the exercise. The main lessons taught by this exercise are that the relative stability of G3P and DHAP determine which will dominate the equilibrium reaction mixture, that the starting ratio of G3P and DHAP has no influence on the final ratio at equilibrium, and that while temperature changes can affect the speed at which equilibrium is attained, they cannot alter the final equilibrium ratio of G3P to DHAP. This "student" model's *Stella* equations and diagram are shown in Appendix B.

Exercise 2 uses a more elaborate model named "Delta G.STM." This model allows the students to vary the standard ΔG of the G3P/DHAP reaction, and teaches the roll that standard ΔG has in determining the equilibrium ratio of reactants to products. The instructor must use the *Stella* application to build this model. The model diagram and equations and a suggestion for the presentation-level interface (consistent with the written instructions in the exercise) are shown in Appendix C. The *Stella* tutorial (which comes with the purchased program) contains detailed directions on how to construct a presentation interface. This model (but not the *Stella* application) was distributed on disk to the ABLE participants in June of 2001.

While this exercise introduces the students to modeling, its main purpose is to teach concepts in reaction equilibrium and thermodynamics, not train the students how to model. Therefore, it uses a highly-directed exercise typical of a lab manual. If the instructor wants to build modeling skill and has more time, it would be possible to give the students the problem of modeling the G3P/DHAP reaction with *Stella*, but not give them these detailed directions. If the instructor is even more ambitious and devoted to modeling as a course objective, *Stella* could be used for independent student modeling projects.

Student Outline

In this exercise, you will use a modeling package called *Stella* to construct a mathematical model of one of the chemical reactions of glycolysis. After exploring the behavior of your model, you will work with more elaborate computer models to help you understand thermodynamics and reaction kinetics.

The Role of Mathematical Modeling

When we try to explain a system, we use our understanding of it to predict its behavior in experiments. Mathematical modeling has an important role in this process for two reasons. First, mathematical models make very precise predictions that can be compared against experimental outcomes. Second, if we describe the system by a set of equations and these equations do produce the observed results, we can be assured that the relationships and behavior contained in the equations *can* produce those results. Of course, this is no guarantee that the exact relationships contained in the equations *are* producing the results, but often answering the "can" question is valuable.

Chemical equilibrium is an important biological phenomenon that is relatively simple to model with difference equations. Complex mathematics is not required.

An Equilibrium Reaction
   At one point in glycolysis, fructose 1,6-bisphosphate splits into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). The overall structure of the reactions is as follows:

fructose 1,6-bisphosphate

G3P ⇄ DHAP

further steps
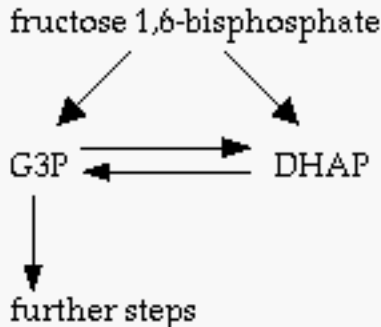
Notice that G3P can transform into DHAP, and DHAP can transform into G3P. This reaction is catalyzed by the enzyme triose phosphate isomerase. Also note that although fructose 1,6-bisphosphate splits into both G3P and DHAP, only G3P continues on to the next reaction. The only way the DHAP can become pyruvate is if it is first transformed into G3P. Therefore, the isomerization reaction between G3P and DHAP is an important part of glycolysis. Because the reaction is simple and contains only one reactant on each side, it is also easy to model.

Concentrations at Equilibrium
   Consider the isolated equilibrium reaction between G3P and DHAP:

$$G3P \leftrightarrow DHAP \qquad\qquad (1)$$

The essence of a reaction at equilibrium is that the forward and backward reaction rates are equal. Equilibrium is attained when the number of G3P molecules transforming into DHAP is equal to the number of DHAP molecules transforming into G3P. However, this does not imply that the equilibrium *concentrations* of G3P and DHAP are equal. These concentrations depend on the relative stabilities of G3P and DHAP, and stabilities can be directly related to their reaction probabilities.

   If G3P and DHAP have the same stability, the probability that a G3P molecule will transform into DHAP is equal to the probability that a DHAP molecule will transform into G3P. In that case, the equilibrium concentrations of G3P and DHAP will be equal.
   However, in fact, G3P is *less stable* than DHAP. At 25°C and 1 M concentrations of both G3P and DHAP, a G3P molecule is 21 times more likely to transform into DHAP than a DHAP molecule is to transform into G3P (Streyer 1995, p. 491). This means that at equilibrium there will be less G3P than DHAP. To understand why, say the number of molecules being transformed back and forth is x per sec at equilibrium. DHAP is stable, has a low probability of transformation, and many molecules must be present to provide x molecules turning into G3P. G3P is unstable, has a high probability of transformation, and only a small number of G3P molecules need be present to provide x molecules/sec turning into DHAP. In fact, the equilibrium ratio is about 21 molecules of _____ (G3P or DHAP) to every molecule of _____ (G3P or DHAP).
   In general, stable molecules have a low probability of reaction and are present at high concentration at equilibrium. Unstable molecules have a high probability of reaction and are only present in low concentrations at equilibrium.

Modeling Reactions with Difference Equations

Using reaction probabilities, chemical reactions can be easily simulated by a system of difference equations. Say that every millisecond, there is a probability of 0.1 that a G3P molecule will form a DHAP molecule, and there is a probability of 0.00475 that a DHAP molecule will form a G3P molecule. Then, each millisecond, the amount of G3P present is the amount present during the last millisecond, plus the net formation or loss of G3P. This net formation or loss rate (ΔG3P) is given by this difference equation:

$$\Delta G3P = 0.00475*DHAP - 0.1*G3P \tag{2}$$

where G3P and DHAP are the concentrations of those molecules. Likewise, the net rate of change of DHAP is given by

$$\Delta DHAP = 0.1*G3P - 0.00475*DHAP \tag{3}$$

In an isolated reaction, the concentrations of G3P and DHAP will automatically adjust themselves until ΔG3P = ΔDHAP. Then G3P and DHAP will be at their equilibrium ratio.

Difference equation modeling is easy with *Stella* software.

Exercise 1. Modeling the G3P-DHAP Reaction with *Stella*

In *Stella*, rather than writing equations, you drag-and-drop icons to construct a diagram of your model. The software automatically produces the equations. After modifying the equations (by supplying constants, for example), you're ready to run your model. A few additional steps, and *Stella* will even graph the results for you. To model the G3P <==> DHAP reaction, follow the following steps:
1. If it's not already running, boot up your computer. Go to the "Start" button and select Programs/Stella Research/Stella.
2. You will see a blank screen on which your model diagram will be constructed. Go to the toolbar at the upper left, and select the rectangle icon as shown below.

Then click somewhere near the middle of your screen and deposit the rectangle there:

Noname 1

A rectangle is the Stella symbol for a "stock," or a quantity of material, like the water in a lake, a population of animals, or a concentration of G3P in a cell.

G3P

3. Rename "Noname 1" as "G3P" and deselect it:

4. Follow the same process to place a "DHAP" stock to the right of the G3P stock:

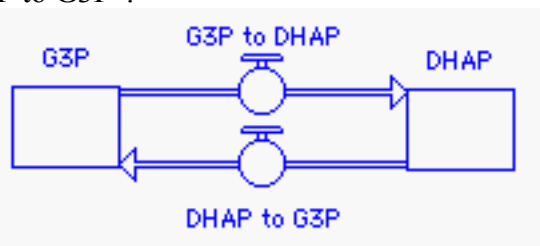G3P   DHAP

5. Now select the "flow" icon ( ) to make a connection between these boxes. Starting well inside the G3P stock, click and hold the left mouse button and drag the arrow over to <u>inside</u> the DHAP stock. The DHAP box will highlight when the connection is made. Then move the flow icon up to the very top of the boxes because we have to have room for another flow icon under it:

G3P   DHAP

Noname 1

   If a cloud appears at the head of the arrow ( ), you have not made the connection to the DHAP box and the flow from G3P will just disappear from the model. If this happens, delete the flow using the Clear command from the Edit menu and try again. Name the flow "G3P to DHAP."

6. Repeat this process, but this time establish a flow from DHAP to G3P, move it to the bottom of the two stocks, and call it "DHAP to G3P":

G3P to DHAP
G3P   DHAP

DHAP to G3P

7. Finally, the amount of material in the G3P and DHAP stocks influences how much flows out of them, so we must indicate an influence of stock size on the outflow rate. Go to the toolbar and select the

   connector icon ( ), and make connections between the stocks and their outflow rates. Move the icon inside the G3P stock, click and hold the left mouse button, and drag the arrow to the inside of the "valve" on the "G3P to DHAP" flow. It will highlight when you've made the connection. <u>Don't</u> connect to the "DHAP to G3P" flow. Then make a connection between the DHAP stock and the "DHAP to G3P" flow. You should have:

G3P to DHAP
G3P   DHAP

DHAP to G3P
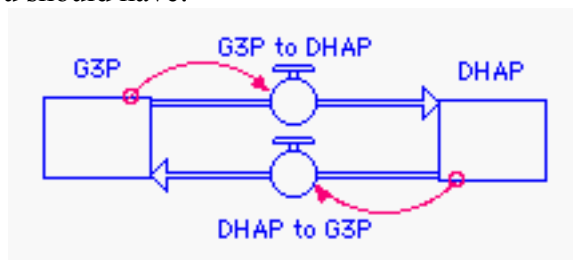
8. This diagram specifies the structure of your first model, but now you must fill in some details of the equations. All this time you have been on the "map/ model" level of Stella, but now you must view

    the equations that Stella has been constructing. Press the down arrow key ( ) at the upper left and you will see the "equation" level:
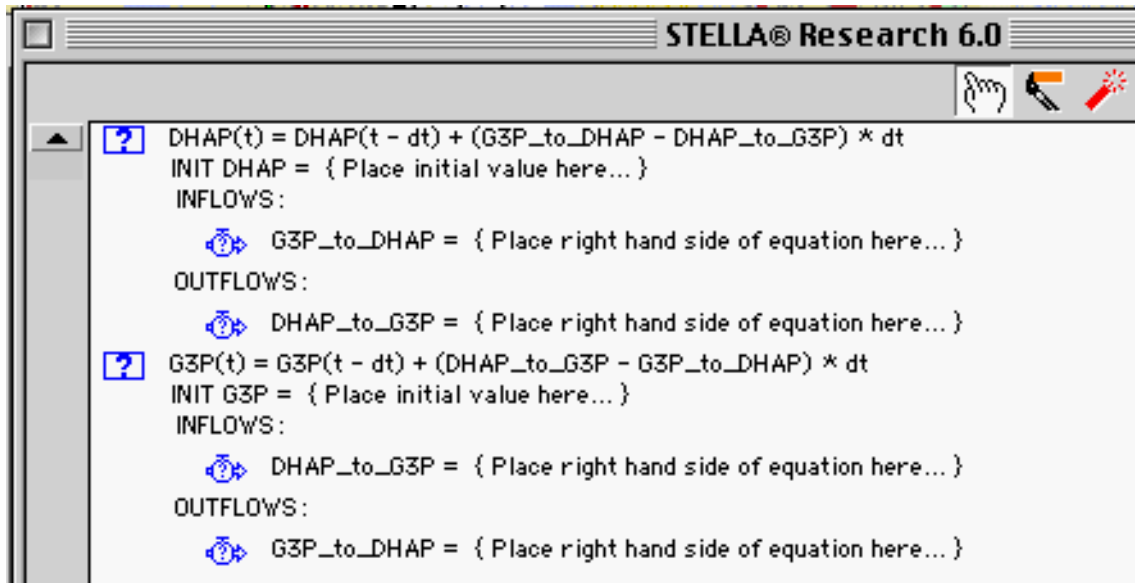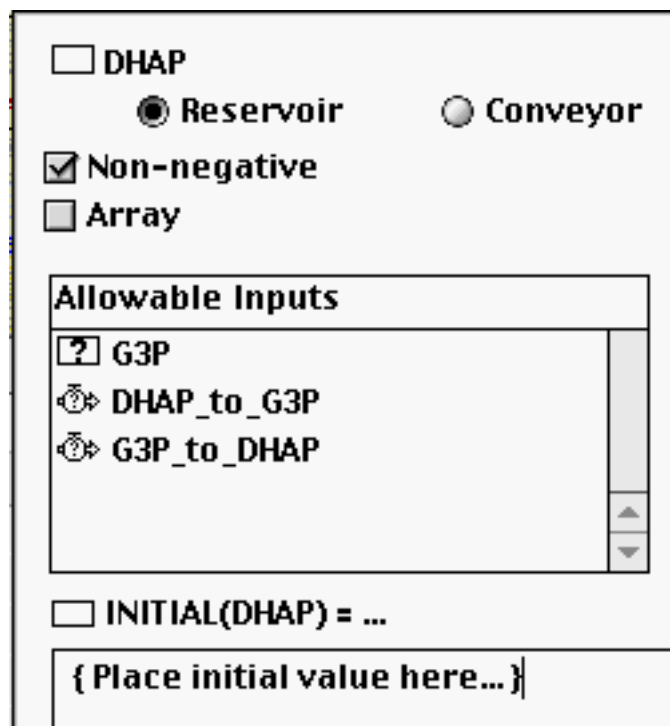

    While these equations look complex, a short examination will disclose that they are very incomplete. There are no initial values for G3P and DHAP, and the flows are merely defined as "{Place right hand side of equation here...}."

9. Double click on the line that says, "INIT DHAP =" and you will see an input screen (as shown at right):

    Replace "{Place initial value here...}"   with 0.5 for 0.5 mM. Click on "OK."
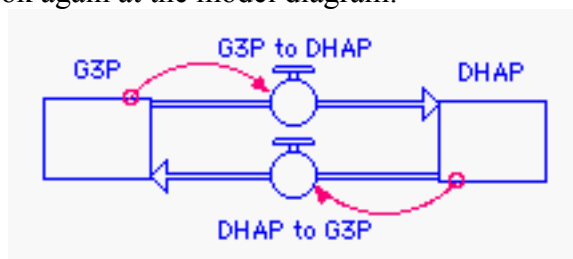
10. Likewise, set the initial value of G3P to   0.5.

11. Double click on either of the two equations that define the flow "G3P_to_DHAP." To understand what we're doing here, look again at the model diagram:


"G3P_to_DHAP" depends on the amount of G3P present (note the curved, thin arrow from G3P to the "valve" that controls the flow rate). For now, we'll say that each G3P molecule has a 0.1 chance/msec of converting to DHAP. Also notice that the "List of Required Inputs" has only G3P on it because the model diagram says this is the only variable that influences the flow rate. Therefore, click once on the "G3P" in the List of Required Inputs and then follow this with "*0.1" as shown below:
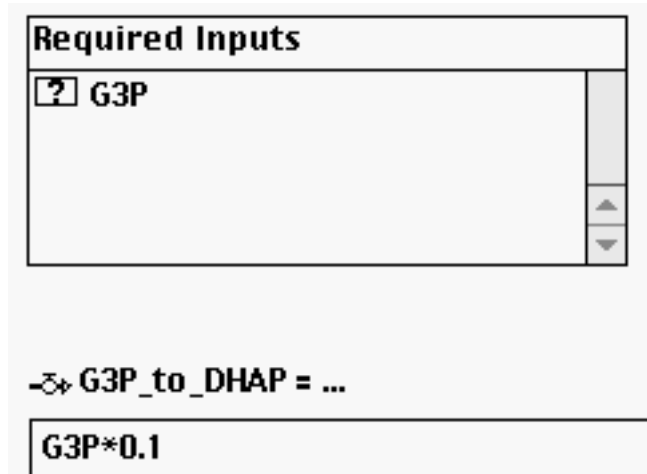
Click on "OK."

12. In a similar way, define the "DHAP_to_G3P" flow as DHAP*0.00475. This "0.00475" is a result of the relative stabilities of the molecules. At 25°C, the probability that a DHAP molecule will transform into a G3P molecule is 0.0475 times as high as the probability that a G3P molecule will transform into a DHAP molecule. We've already defined the reaction probability for G3P as 0.1/msec. Therefore, the DHAP reaction probability is 0.1*0.0475 = 0.00475/msec.
Note that although you've only filled in the constants for one "G3P_to_DHAP" equation and one "DHAP_to_G3P" equation, *Stella* has filled in the other two equations for you. The inflow for one stock is automatically the outflow for the other stock.

13. Now you're finished with the first stage of your model building, but you still have to specify what kind of output you want. A graph is the best output here. Press the up arrow at the upper right corner

of the screen and go back to the model diagram. Find the graph icon ( ) and place it on the model diagram screen somewhere under the model. Now you must specify the x-axis and y-axis range of this graph. Double-click on the graph icon to show a blank graph.

a) Under the "Run" menu, select "Time Specs." Indicate that you want the time to run from 0 to 50. Change the time unit to "Other" and write in "msec." Click OK. The graph will still have an x-axis that runs from 0 to 12, but don't worry about this. It will change when you run the model.

b) To establish the y-axis, double-click on the blank graph. You should see the screen below.
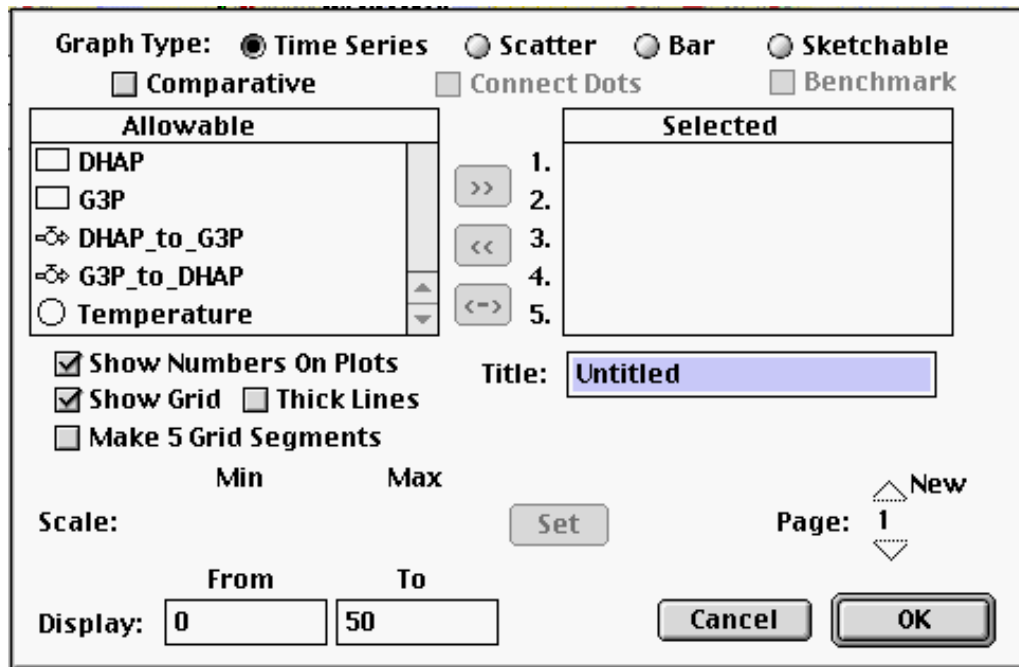
You must tell the graph to plot G3P and DHAP on its y-axis. Select DHAP and G3P on the left side and click the (>>) button. Those stocks will appear in the "Selected" column. Then select both G3P and DHAP in the "Selected" column. Click on the ⬍ icons to their right. When these change to the ⬍ icon, you may type in the y-axis range in the boxes marked "Scale." Enter a range of 0-1. Click on the "Set" button and then on the "OK" button.

14. Your model is now ready to run. However, before you run it, make a prediction:
    ♦ If the probability of G3P --> DHAP is 0.1/msec, and the probability of DHAP --> G3P is only 0.00475/msec, which will be more abundant at equilibrium, G3P or DHAP?

    Write your answer here _____ and then select "Run" from the "Run" menu, and see if you were right. Note that both curves are labeled and color-coded, so it is easy to tell one from the other.

15. You started the concentrations of both G3P and DHAP at 0.5 mM, a 1:1 ratio. Would the final equilibrium be different if you varied the starting ratio of G3P and DHAP? Write your prediction

    here _____ (yes or no) and proceed to vary the concentrations. Press the ⬍ down arrow key at the upper left and go back to the equation level. Following the procedure in step 9, change "INIT G3P" to zero, and then change "INIT DHAP" to 1.0. Go back to the model level (up arrow), double-click on the graph icon to get the graph back, and run the model. Are the relative amounts of G3P

and DHAP at equilibrium different from the 1:1 case? In a similar way, go back to the equation level and change the starting concentrations to 1.0 for G3P and zero for DHAP. Run the model by selecting "Run" from the Run menu. Does the equilibrium ratio change?

◆   What is the effect of initial concentrations on the ratio of products to reactants at equilibrium?

_____

◆   Why does this outcome occur? Hint: What determines relative equilibrium concentrations, and is this

influenced by initial concentrations? _____

16. The relative stability of reactants and products determines where the equilibrium point of a reaction lies, but it does not determine how fast that equilbrium is attained. The G3P <--> DHAP reaction is governed by an enzyme, and one of the major influences on its rate of activity is temperature. *You will now incorporate the effect of temperature into your model.*

17. You should be on the map/model level. Click away the graph to reveal the model diagram. Go to the toolbar and select the circle, the *Stella* symbol for a "converter." A converter is usually some condition that influences a rate. Deposit the converter somewhere to the right or left of your model. Label it "Temperature." Then draw connector arrows from temperature to the two valves. Your final diagram should look like this:
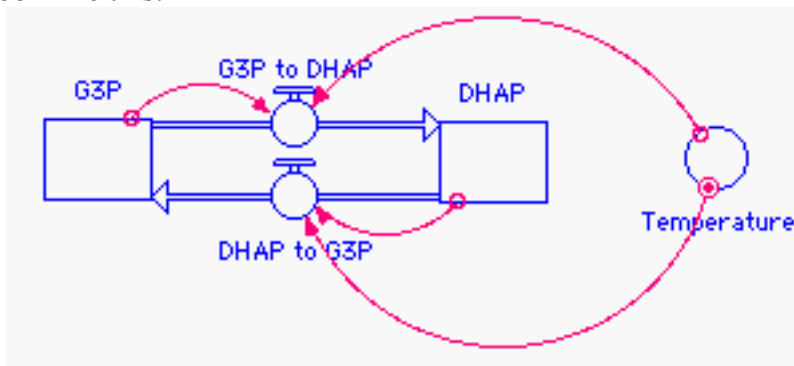
18. The red arrows indicate that temperature influences reaction rates, but we haven't said how. Within small ranges around room temperature, reaction rates tend to double for each 10° rise in temperature and tend to fall by half if the temperature drops by 10°. Thus, we say $Q_{10} = 2$. Also, 25° is often used as a standard temperature in biochemistry. Go to the equation level and double-click on the temperature line. Enter 25 as the initial temperature value. Click OK.

19. Now we must quantify the effect of temperature on the reaction rates. Click on the line that defines "G3P_to_DHAP" and change it to the following:

$$\text{G3P\_to\_DHAP} = ...$$
$$\text{G3P*0.1*2\^((Temperature} - 25)/10)$$

Be sure to get the parentheses in the right spots. If temperature changes from 25° to 35°, can you see how this will double the reaction rate by multiplying the rate by 2^1, or 2?

20. Make the same change to the line defining DHAP_to_G3P. That is, multiply by

$$2\char`^((\text{Temperature} - 25)/10).$$

21. Bring the initial values of DHAP and G3P back to 0.5.

22. Now go back to the model level, double-click on the graph icon to bring up the graph, and test the effect of temperature:

   a) With the temperature at 25°, run the model. It should give you the same results as before.

   b) Go the equation level and change the value of temperature to 4° (common in refrigerators). Go back to the map/model level, call up the graph, and run the model. How are the results different from the 25° case?

   c) Change the temperature to 40° and run the model again. How are these results different from the 25° case?

23. Answer the following questions:

   a) In this model, does temperature influence the relative concentrations of G3P and DHAP once

   equilibrium is attained? _____ (yes or no)

   b) What *does* temperature influence? _____

   c) Why does temperature have this effect? _____

Gibbs Free Energy Change and Reaction Equilibria
   The relative size of forward and backward reaction rates of a reaction is determined by a quantity called the Gibbs free energy change of the reaction (ΔG), measured in kilocalories per mole. This is a complex thermodynamic quantity that includes both the energy released or absorbed by the reaction and the change in the amount of order of the reactants.
   For example, when a cell oxidizes the sugar glucose into CO_2 and water, ΔG is said to be negative because the glucose was a complex molecule with much energy in its bonds, and its transformation into CO_2 and water releases energy, and produces disordered, simpler molecules. That is, bond energy has decreased and order has decreased, so the ΔG of the reaction is negative. Reactions like this are called *exergonic* because they release energy.
   On the other hand, when a plant uses the energy of the sun to photosynthesize CO_2 and water into sugar, the reaction is absorbing energy and transforming disordered, simple, low-energy molecules into large and complex ones that contain much energy in their bonds. The energy content of the molecules is going up and their order is going up, and the ΔG of the reaction is positive. These reactions are called *endergonic* because they absorb energy.
   One of the most important principles of biochemistry is that *the forward rate will be greater than the backward rate if ΔG is negative*. Reactions with negative ΔG are said to be *spontaneous*. On the other hand, *if ΔG is positive, the backward rate will be faster*, and the reaction is said to be *nonspontaneous*. Note that this refers only to the direction of the reaction, not its speed. A "spontaneous" reaction (like the oxidation of glucose in air at room temperature) could be so slow that it might never occur to any practical extent.

ΔG changes with the relative concentrations of reactants and products. In order to determine the relative size of the forward and backward rates, we should compare the rates when the reactant and the product have *equal concentrations*. ΔG when all reactants are at a concentration of 1 mole/liter is called standard free energy change, and is abbreviated ΔG°. The ratio of the forward reaction rate to the backward reaction rate is called the equilibrium constant, or K_{eq}:

$$K_{eq} = \text{forward rate/backward rate} \qquad\qquad (4)$$

K_{eq} is related to ΔG° by the equation

$$K_{eq} = 10^{(-\Delta G°/(2.303\ RT))} \qquad\qquad (5)$$

where R is the gas constant (1.98×10^{-3}), and T is the Kelvin temperature (the Celsius temperature + 273) (Streyer 1995, p. 187). For example, if ΔG° = -1 and the temperature is 25° C (298 K), then

$$K_{eq} = 10^{(1/(2.303*\ 0.00198*298))} = 10^{0.7359} = 5.44 \qquad\qquad (6)$$

Therefore, the ratio of the forward and backward rates in this case will be 5.44, and the reaction will go forward if reactants and products are at equal concentrations. In general, by looking at the equation defining K_{eq}, we can see that a negative ΔG° will produce a positive exponent and a K_{eq} greater than 1, and a positive ΔG° will produce a negative exponent and a K_{eq} less than 1.

Note that from now on, we will be talking about reactions with a stated direction. "DHAP <==> G3P" has no clear reactants and products, and we cannot specify its ΔG^0 for that reason. However, "DHAP --> G3P" has DHAP as its reactant, G3P as its product, and its ΔG^0 is 1.8 at 25° C. The ΔG^0 of the G3P --> DHAP reaction (the reverse reaction) is -1.8 under those same conditions.

In addition, knowing the ratio of the rates does not tell us the magnitude of the rates. Knowing that K_{eq} = 5.44 in the case above does not tell us whether the backward rate and the forward rate are 0.1 and 0.544, or 0.001 and 0.00544. We know that the reaction will move forward, but we don't know how fast. The speed of a reaction is related to other factors, especially enzyme concentration and temperature, but not to ΔG°.

Exercise 2. Exploring the Influence of ΔG^0 on a Reaction

1. Make sure you have finished all the exercises above. Then close your model of the DHAP/G3P equilibrium, and don't save your work. Using the "File" menu of Stella, open the "Models" folder, and then the file "Delta G.STM."

2. This file uses the "presentation" level of Stella for more rapid use, but it is very similar to the model you constructed before. When the model opens, you should see that:
   a) the "G3P" dial should be set to 0.5 mM (which also sets DHAP to 0.5 mM);
   b) the "Stan Delta G" slider should be set on 1.8;
   c) the temperature slider should be set to 25°.

3. Press the "Run" button to run the model under these default conditions, and verify that the results are the same as what you observed with your model.

4. Leave "Stan Delta G" alone but vary the temperature and verify that the results are very similar to what your previous model produced.

5. By turning the G3P-DHAP dial, you can produce initial reaction mixtures that vary from all G3P to all DHAP and anywhere in between. Here, the initial DHAP concentration is always 1 - G3P. Vary the initial concentration of G3P and verify that the results are similar to your observations with your earlier model.

6. Press the small "U" button on the temperature slider to set the temperature back to 25°, and on the G3P dial to bring G3P back to 0.5 mM. Now, make some predictions:

   a) The "Stan Delta G" slider shows the ΔG^0 of the *DHAP --> G3P reaction*. If the slider is moved

      from +1.8 to +4.0, the effect on the reaction will be to make _____ (DHAP or G3P) more

      dominant in the equilibrium reaction mixture.

   b) If the slider were moved to some small positive value like +0.2, the equilbrium reaction mixture

      would consist of _____.

   c) If the slider were moved to exactly 0, the equilbrium reaction mixture would consist of

      _____.

   d) If the slider were moved to a value like -4.0, the equilbrium reaction mixture would consist of

      _____.

7. Test all these predictions. Were you right?

8. Reactions with positive ΔG^0 are said to be "nonspontaneous," but this does not mean that they cannot occur.

   a) Set G3P and DHAP to 0.5 mM, temperature to 25°, and ΔG^0 to some small, positive value like 1. Run the model. Does the reaction move forward (towards formation of more G3P)? Why or why not?

      _____

   b) Set G3P to zero and DHAP to 1.0 mM. Keep the ΔG^0 at the positive value you used before. Run the model again. Does the reaction move forward (towards formation of more G3P)? Why or why not?

      _____

9. Sketch the rough time course of the *G3P* concentration under each of the following conditions on the graphs depicted below. In all cases, the initial [DHAP] = 1 - initial [G3P]. Therefore, if [G3P] = 0.5, then [DHAP] = 0.5. In addition, ΔG^0 refers to the *DHAP --> G3P* reaction. (Hint: you should certainly be able to figure out the first one, and after that, you should be able to solve the rest by asking what has changed from the first one, and what effect that change will have.)
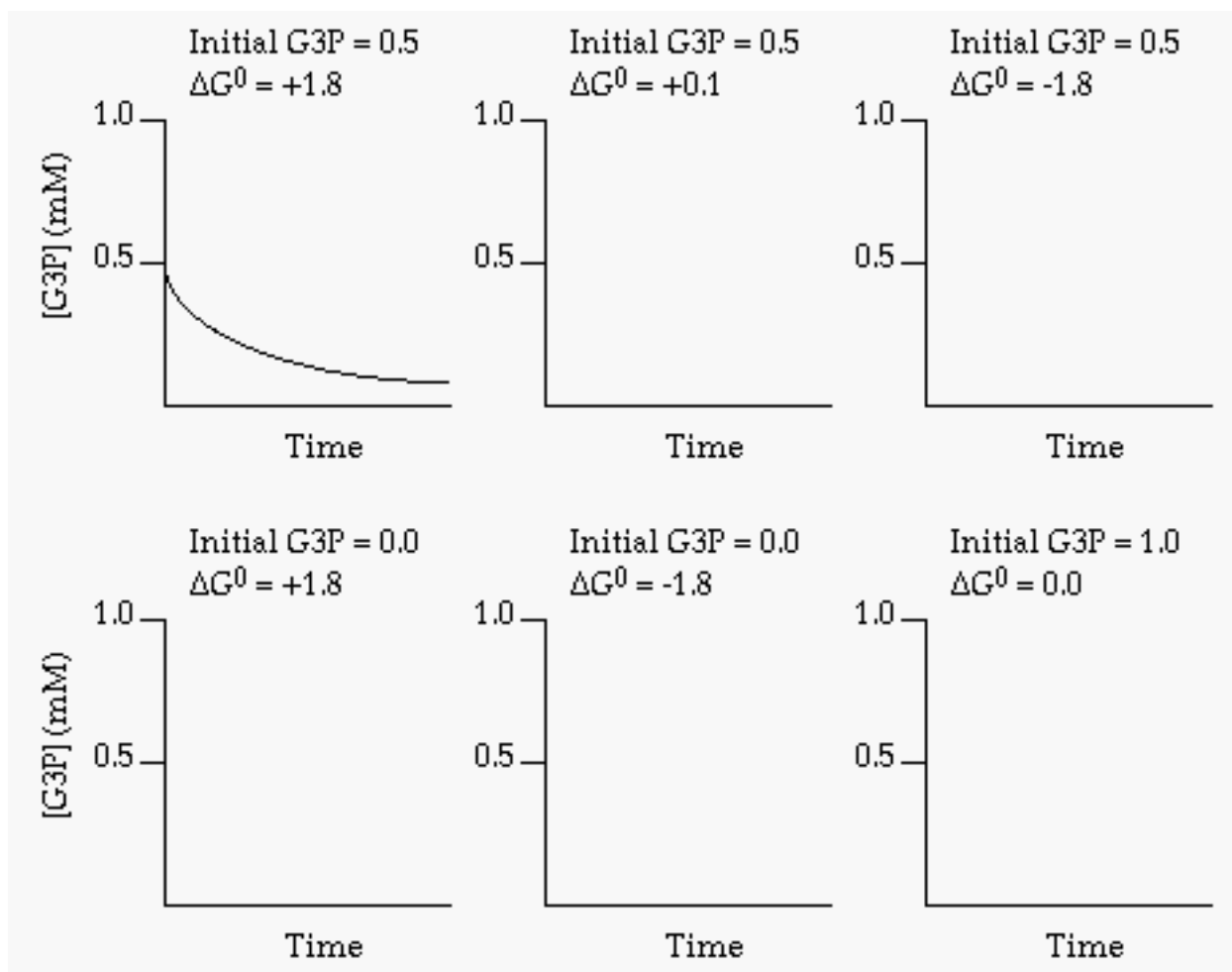
10. Test your predictions with the Delta G.STM model.

Acknowledgements

Literature Cited

Streyer, L. 1995. Biochemistry. Fourth Edition, W. H. Freeman and Company, New York, 1064 pages. [ISBN 0-7167-2009-4]

Appendix A
Answers to Questions

p. 258   There will be 21 molecules of DHAP for every molecule of G3P because DHAP is more stable and has a much lower probability of reaction than G3P.

p. 263   There will be more DHAP because DHAP is more stable and has a lower probability of reaction.

No, there will be no difference in the final ratio of DHAP to G3P, no matter what the starting ratio is. Even if you start with all of one and none of the other, the reactant that is present will rapidly form some of the reactant that is not present. Once the mixture reaches the equilibrium ratio of reactants to products, the ratio will remain unchanged because equilibrium is the point at which the forward and backward reaction rates are equal.

p. 264   There is no effect of initial concentrations on the final ratio of products to reactants. The equilibrium ratio is determined by the relative stabilities of the two molecules, and is not affected by concentration. Note that this is not saying that the reaction *rates* are not affected by concentration, just that the *final ratio* of G3P to DHAP is not affected by initial concentrations.

p. 265   No, temperature does not affect the equilibrium ratio of DHAP to G3P. It does affect the speed with which the equilibrium ratio is attained, however. Temperature has this effect due to normal kinetic processes-- warmer molecules move faster and react more quickly.

(Actually, even the equilibrium ratio is affected slightly by temperature, but the simple model above does not include this effect.)

p. 267   If the ΔG^0 of the DHAP --> G3P reaction is made more positive, the reaction as written will be even less spontaneous and the reaction mixture will be more dominated by DHAP at equilibrium.

If the ΔG^0 of the DHAP --> G3P reaction is made only slightly positive, DHAP will dominate the reaction mixture, but only by a slight margin.

If the ΔG^0 of the DHAP --> G3P reaction is zero, there will be exactly as much DHAP as G3P at equilibrium.

If the ΔG^0 of the DHAP --> G3P reaction is negative, the reaction will be highly spontaneous and the reaction mixture will be dominated by G3P because now G3P is the more stable reactant.

With ΔG^0 at a positive value, the DHAP ---> G3P reaction is nonspontaneous and will move to a higher concentration of DHAP than G3P. In other words, there will be no net formation of G3P. This does not mean that the DHAP --> G3P reaction is not occurring, just that it is not as fast as the opposite reaction.

p. 267   Yes, now the reaction does move forward and produce more G3P than was present at the start, even though ΔG^0 is still positive. This happens because at the start, there is plenty of DHAP to form G3P, but no G3P to back-react into DHAP. This situation is only temporary. Very soon, a small amount of G3P forms, and the rate of the G3P --> DHAP reaction becomes equal to the rate of the DHAP --> G3P reaction. After this, there is no more net formation of G3P. This is why it is so important for the student to grasp that "nonspontaneous" doesn't mean "can't happen." Even a "nonspontaneous" reaction with a positive ΔG^0 will move forward if there is a high concentration of reactants and a low concentration of products. Remember that a positive ΔG^0 refers to ΔG with equal concentrations of reactants and products.
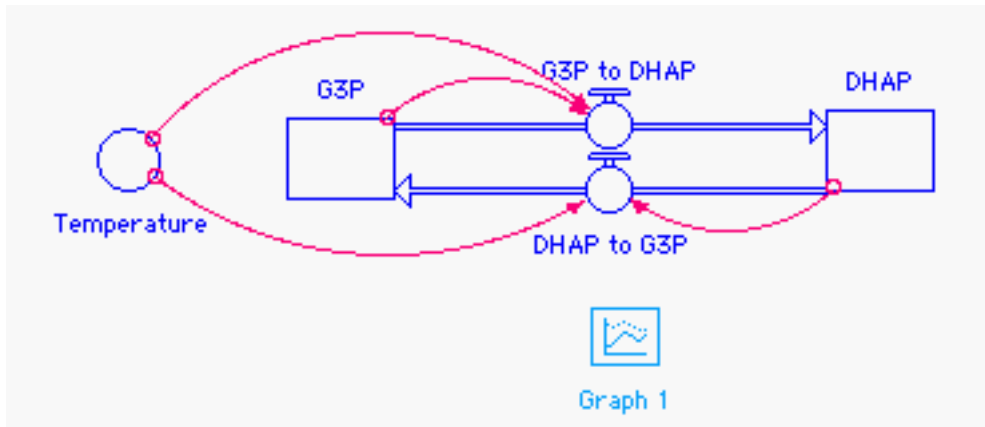
Graphical answers appear below:
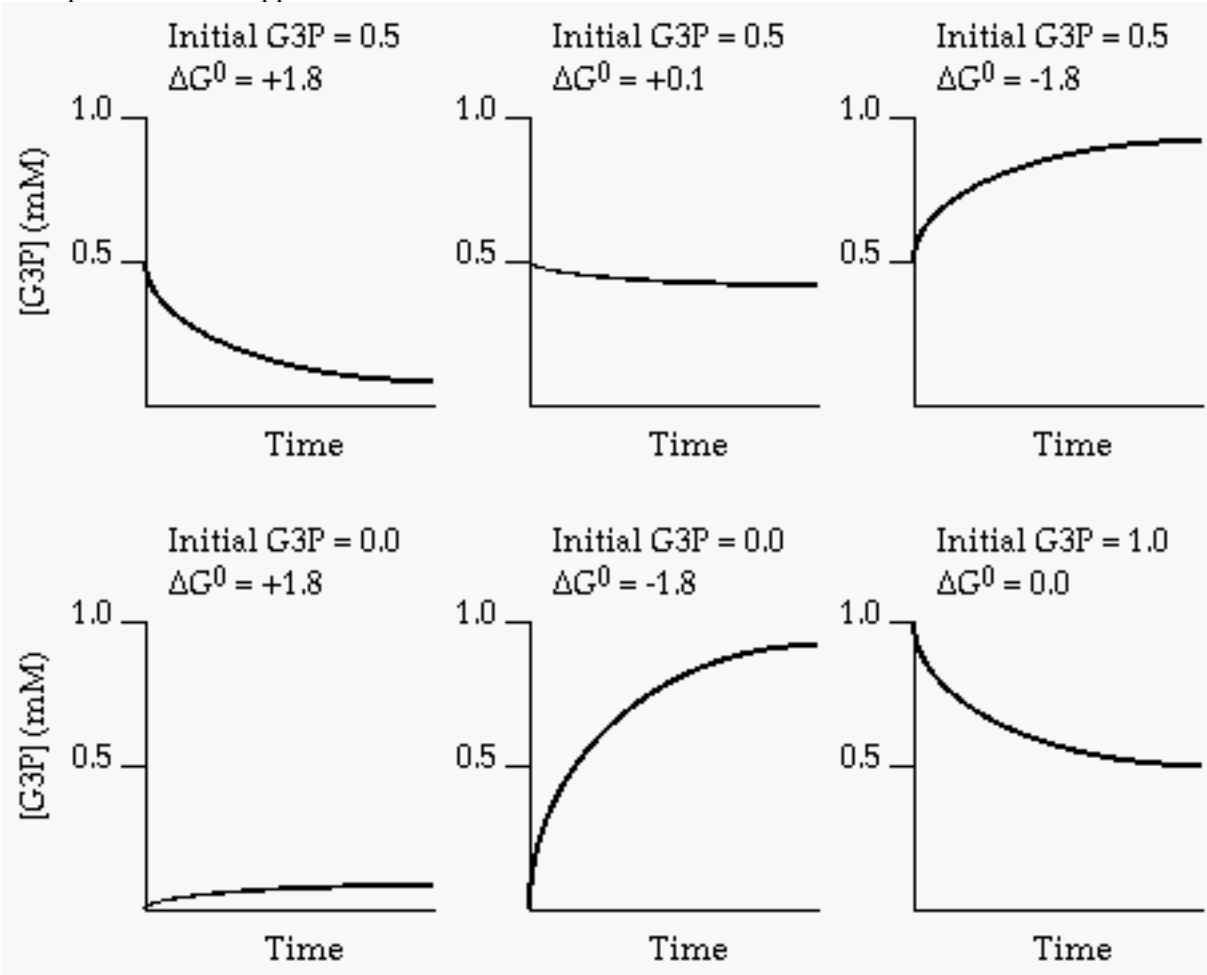
Appendix B
Diagram and Equations for the "Student" Model

This model is constructed by the students using the directions in the exercise.

DHAP(t) = DHAP(t - dt) + (G3P_to_DHAP - DHAP_to_G3P) * dt

INIT DHAP = 0.5
INFLOWS:
        G3P_to_DHAP = G3P*0.1*2^((Temperature-25)/10)
OUTFLOWS:
        DHAP_to_G3P = DHAP*0.00474*2^((Temperature-25)/10)
        G3P(t) = G3P(t - dt) + (DHAP_to_G3P - G3P_to_DHAP) * dt

INIT G3P = 0.5
INFLOWS:
        DHAP_to_G3P = DHAP*0.00474*2^((Temperature-25)/10)
OUTFLOWS:
        G3P_to_DHAP = G3P*0.1*2^((Temperature-25)/10)

Temperature = 25

Appendix C
Diagram, Equations and Presentation Suggestions for the "Delta G" Model

If the instructor wishes to use the "Delta G" model, he/she must build it using *Stella* and make it available to the students. The diagram below and the equations are solely for the use of the instructor, since the students only see the "presentation" level of this model.

The diagram:
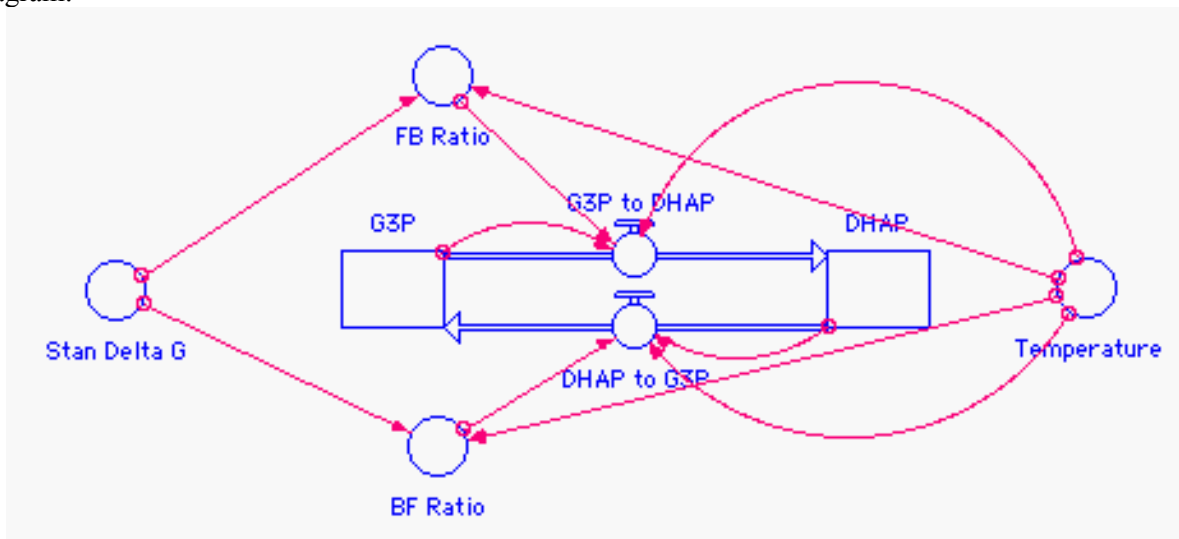
The equations:

"FB_Ratio" means the ratio of the forward rate to the backward rate in the reaction DHAP --> G3P, and "BF_Ratio" means the ratio of the backward rate to the forward rate in the same reaction. These ratios are both functions of ΔG°.

DHAP(t) = DHAP(t - dt) + (G3P_to_DHAP - DHAP_to_G3P) * dt
       INIT DHAP = 1 - G3P
INFLOWS:
       G3P_to_DHAP = G3P*0.1*FB_Ratio*2^((Temperature - 25)/10)
OUTFLOWS:
       DHAP_to_G3P = DHAP*0.1*BF_Ratio*2^((Temperature - 25)/10)

G3P(t) = G3P(t - dt) + (DHAP_to_G3P - G3P_to_DHAP) * dt
       INIT G3P = 0.5

INFLOWS:
       DHAP_to_G3P = DHAP*0.1*BF_Ratio*2^((Temperature - 25)/10)

OUTFLOWS:
       G3P_to_DHAP = G3P*0.1*FB_Ratio*2^((Temperature - 25)/10)

BF_Ratio = if (Stan_Delta_G >= 0) then EXP(-Stan_Delta_G/((1.99E-3*(273
       + Temperature)))) else 1
FB_Ratio = if (Stan_Delta_G >= 0) then 1 else EXP(Stan_Delta_G/((1.99E-
       3*(273 + Temperature))))

Stan_Delta_G = 1.8
Temperature = 25

The presentation level of this model should be constructed using the directions contained in the *Stella* tutorial. To be consistent with the written directions, the instructor should add a run button and sliders. The run button is a dial that allows the user to dial the G3P concentration from 0 to 1 mM (note that the initial DHAP concentration is always 1 – the G3P concentration). Sliders can set the standard delta G of the reaction (from –5 to +5) and the temperature (from 0° to 50° C). The presentation level should look like this:
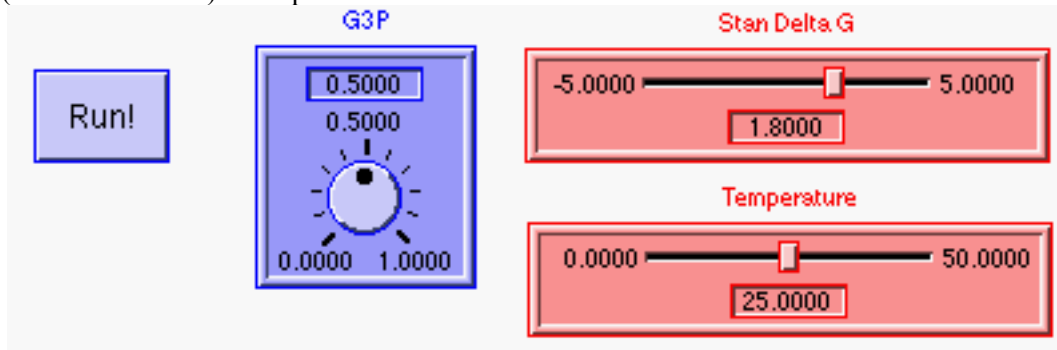
This should be followed by a graph on the presentation level that shows DHAP and G3P, both graphed from 0 to 1 mM.

Appendix D
The "Basic" vs. "Research" Versions of *Stella*

The differences between the Basic and Research versions of *Stella* are not easy to determine, either on the HPS website or in the *Stella* documentation. The description below was e-mailed to Kosinski from Debra Gonzales of HPS in April of 2001.

> The metabolic models in this exercise can easily be handled by the Basic version. The Research version features like Queues and Ovens would mainly be used in discrete modeling situations like simulating the arrival and processing of patients at an emergency room where there are multiple "stops" that a patient makes and it is important to find bottlenecks and accurately predict the peak loads that the system can handle. The other Research version features below are useful for managing very complicated models. In my opinion, these additional features are handy but not necessary for most student modeling in biology.
>
> Ms. Gonzales' e-mail

The STELLA Basic software provides solid, basic modeling and simulation capability. It allows a model builder to:

- create a simplified picture of the situation being studied;
- facilitate a top-down approach to model construction;
- use the four basic building blocks (stocks, flows, converters, and connectors) to represent any process;
- use the built-in functions to facilitate incorporation of mathematical, statistical and logical operations.

The STELLA Research version provides all of the above. In addition, the Research version gives a model builder the ability to develop more sophisticated or detailed models using the following additional features:

- **Sub-Models**: Sub-Models provide an excellent mechanism for managing diagram complexity. Sub-Models enable you to "drill down" into various stages of a process, to represent sub-processes with a greater degree of detail. By managing detail, sub-models can keep you (as well as the users of your models) in control of the complexity.
- **Space Compression Object**: Space Compression Object is another mechanism for managing diagram complexity. It enables you to compress space associated with the detailed logic of a model. This space can be selectively shown on the diagram, or hidden from view.
- **Queues & Ovens**: Queues and Ovens are advanced typed of stocks. Think of a queue as a line of items awaiting entry into some process or activity (e.g., grocery store checkout or airport ticket counter line). Think of an oven as a processor of discrete batches of items. The oven opens its doors; fills; bakes its contents for a period of time; then unloads in an instant.
- **Cycle-time**: The Cycle-time functionality enables you to generate and collect a variety of useful metrics regarding the amount of time that material spends "in process" as it moves through a conserved flow chain. Such metrics can be valuable in a variety of modeling contexts. In particular, cycle time metrics can be of considerable value when you are engaged in a process improvement effort.
- **Arrays**: Arrays offer an extremely powerful way to manage the visual complexity of highly disaggregated models. With a single building block representation, you can model multiple parallel structures. For example, one arrayed stock can represent predators distributed across several different regions and species groups.