

# APPENDIX I

## LAB INVESTIGATIONS AND EXERCISES

Labs are an important part of your course work because they are a way to help you apply the themes and concepts you are studying in class to biological processes. Some important goals of lab exercises include helping you to: make systematic observations of the natural world; learn how to use scientific apparatus; make predictions and hypotheses; gather, analyze and interpret data; communicate experimental results; and pose questions for further investigation. They require you to identify assumptions, use critical and logical thinking, and consider alternative explanations.

As an example, suppose you are a member of a group that is investigating the effects of different amounts of nitrogen on the growth of a particular species of plant. How would your investigation proceed to meet the goals given above?

### Identify Questions and Concepts

Your group would first define and agree on the problem to be investigated. Questions such as "What percentage of nitrogen would cause the plants to grow the tallest?" help to focus the investigation on one particular aspect of the plant's response to the added nitrogen. Next, you would develop several hypotheses, or educated guesses, that describe the plant's responses. Hypotheses are presented in an if... then... format; for example, "If plants are given additional nitrogen then they will grow taller than plants that receive no additional nitrogen."

In developing a good hypothesis it might be necessary to conduct a review of textbooks or other resources. For example, a review would help clarify what role nitrogen plays in plant growth.

### Design and Conduct the Investigation

Using the hypothesis, the group would next design an experiment to compare the effects of different amounts of nitrogen added to the plants. It would need to identify constants (controlled variables), those factors that remain consistent throughout the experiment. Constants would be such factors as the species of plant used, the volume of nitrogen applied to the plants, the amount of light each plant receives, room temperature, and the form in which the nitrogen is delivered. Also, the group would need to identify the variables. An independent variable is a factor that is purposefully changed, in this case the percentages of nitrogen. There might be several levels of the independent variable, for example, nitrogen concentrations of 1%, 3%, 5%, 7%, and 10%. A dependent variable is the variable that responds to the change in the independent variable. In this experiment, the height of the plant would be the dependent variable.

The experiment would also need to have a control, a group of plants that receive no additional nitrogen (in other words, a group in which the independent variable is not changed). Controls are a way to detect or measure the influence of unanticipated factors, and they establish a base line for comparing the experimental effects to no treatment.

Finally, the group would need to include repeated trials (replication) in the experimental design. These repeated trials reduce the probability that a change occurred because of chance, some random factor, or because of individual variations within the plants.

**Use Appropriate Technology**

An important component of any scientific investigation is the use of appropriate technology that aids in the collection, analysis, and presentation of data. The group could use computers and spreadsheets to organize the raw data and summarize it by using statistical analysis. Graphs and charts could be produced that would clearly and concisely communicate the results.

**Formulate and Revise Explanations and Communicate the Results**

The final task for the group would be to analyze the results and communicate them in an accurate and effective manner. This would be accomplished by constructing a reasoned argument, careful and logical writing, and the use of charts and graphs. The discussion might include other plausible explanations, but these would need to clearly identify and support the best explanation of the observed results. Other components of the report might include an explanation of the statistical analysis, an oral presentation, and the appropriate response to critical comments. Discussion questions are an important way of stimulating and directing dialogue about a topic or concept.

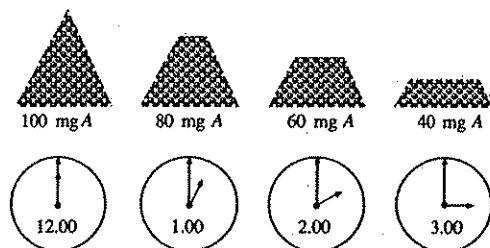
**Investigate Further**

Lab exercises often lead to other questions or suggest alternative methods for performing the investigation. For example, using the goals and steps given above, the group might wish to investigate whether nitrates, nitrites, or ammonia are most efficiently used by the plant; or they might choose to determine if delivery to the roots or the leaves is the best way to get the nitrogen into the plant.

# APPENDIX II CONSTRUCTING LINE GRAPHS\*

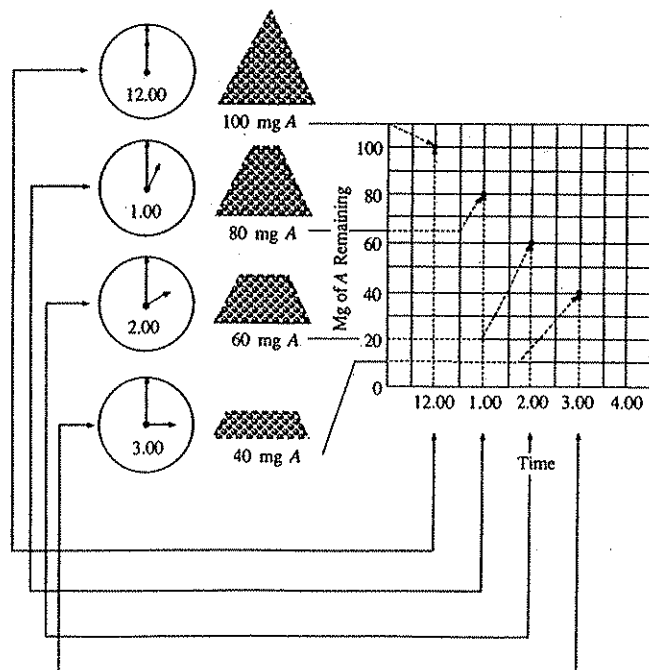
Suppose we are studying some chemical reaction in which a substance, A, is being used up. We begin with a large quantity (100 mg) of A, and we measure in some way how much A is left after different times. The results of such an experiment might be presented pictorially like this:

Figure A.1



This is the kind of picture graph that you often see in newspapers. This information can be presented much more simply on a graph — a line graph is permissible — because our experience tells us that when A is disappearing in a chemical reaction, it is disappearing more or less smoothly and will not suddenly reappear again. In other words, the progress of a chemical reaction is a continuous process, and because time is a continuous process it is permissible to relate the two kinds of information to one another on a line graph. The procedure for constructing the line graph is shown in Figure A.2.

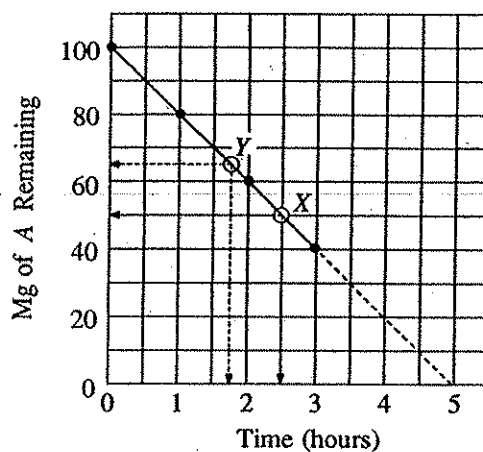
Figure A.2



\* Based on a handout by Dr. Mary Stiller, Purdue University.

It should be clear from the diagram that each point corresponds both to a particular measurement of the amount of *A* remaining and to the particular time at which that amount remained. (A heavy dot is made opposite both of these two related quantities.) When all the measurements have been recorded in this way, we connect the dots with a line, shown in Figure A.3. (Figures A.21–A.23 explain when to connect the data points.)

Figure A.3



It should be clear by looking at our graph that the only measurements we actually made are those indicated by the dots. However, because the information on both scales of the graph is assumed to be continuous, we can use the graph to find out how much *A* would have been found if we had made our measurements at some other time, say 2.5 hours. We merely locate the line that corresponds to 2.5 hours on our time scale and follow it up until it crosses our line graph at the point *X*; then we look opposite *X* to the “Mg of *A* Remaining” scale, and read off 50 mg. We conclude, then, that if we had made a measurement at 2.5 hours, we would have found 50 mg of *A* left. In a similar way, we can find out from our graph at what time a given amount of *A*, say 65 mg, would be left. We have merely to find the line that represents 65 mg on the vertical scale and follow it across until it cuts the line graph at point *Y*. Then we see 1.75 hours on the “Time” scale opposite *Y*. This tells us that had we wished to stop the reaction with 65 mg of *A* remaining, we would have had to do so after 1.75 hours.

You will notice that part of the graph has been drawn with a broken line. In making a line graph we are properly allowed to connect only the points representing our actual measurements. It is possible that measurements made after 3 hours will give points that will fall on the broken-line extension of the graph, but this is not necessarily so. In fact, the reaction may begin to slow up perceptibly, so that much less *A* is used up in the fourth hour than in the third hour. Not having made any measurements during the fourth hour, we cannot tell, and we confess our ignorance quite openly by means of the broken line. The broken line portion of the graph is called an **extrapolation**, because it goes beyond our actual experience with this particular reaction. Between any two of our measured points it seems fairly safe to assume that the reaction is proceeding steadily, and this is called an **interpolation**. Interpolations can only be made between measured points on a graph; beyond the measured points we must extrapolate. We know that the amount of *A* remaining after 4 hours is somewhere between 0 and 40 mg. The amount indicated by the broken line on the graph, 20 mg, is only a logical guess.

Unfortunately, it sometimes happens that even professionals take this sort of limitation of line graphs for granted and do not confess, by means of a broken line, the places where they are just guessing. Therefore, it is up to readers of the graph to notice where the last actual measurement was made and use their own judgment about the extrapolated part. Perhaps the extrapolated part fits

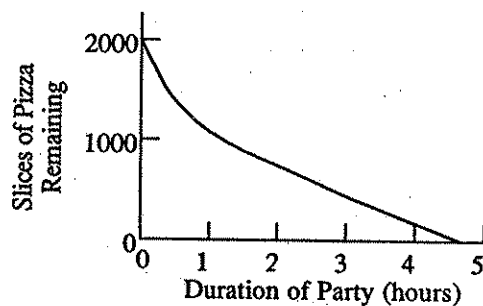
quite well with the reader's own experience of this or a similar reaction, and he or she is quite willing to go along with the author's extrapolation. On the other hand, the reader may be interested only in the early part of the graph and be indifferent to what the author does with the rest of it. It may also be that the reader knows that the graph begins to flatten out after 3 hours and so disagrees with the author. The point is that we, the readers, must be aware of what part of the graph is extrapolated, that is, predicted, from the shape of the graph up to the time when the measurements were stopped. Hence, you must clearly indicate on a line graph the points that you actually measured. Regardless of what predictions or conclusions *you* want to make about the graph, you *must* give the reader the liberty of disagreeing with you. Therefore, it is very improper to construct a line graph consisting of an unbroken line without indicating the experimentally determined points.

### Basic Requirements for a Good Graph

The following procedure applies primarily to graphs of experimental data that are going to be presented for critical evaluation. It does not apply to the kind of rough sketch that we often use for purposes of illustration.

Every graph presented for serious consideration should have a good **title** that tells what the graph is about. Notice that we need more than just a title; we need a *good* title. Before we try to make a good title, let us look at an example and try to decide what kind of title is a useful one. Look at Figure A.4.

Figure A.4



If you like pizza, it might be very useful to know when this party is being held. Without a title, you cannot tell even whether the graph refers to any particular party at all. It might represent average figures for all the parties held last year, or it might represent the expected figures for a party that is going to be held tonight. Let us suppose that these data refer to a study party given by AP Biology students on March 9. Here, then, are some possible titles:

- (a) The APs Have a Party
- (b) Pizza Rules! Enjoy it with AP
- (c) An AP Biofeast!

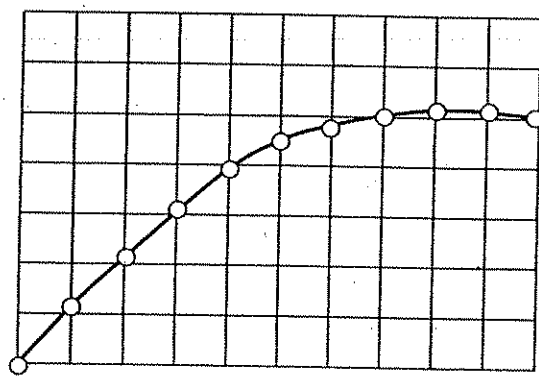
None of those titles are especially useful or informative, because none of them tell what the graph is all about. Now look at these two titles:

- (d) Anticipated Consumption of Slices of Pizza at the AP Biology Party, March 9
- (e) Anticipated Consumption of Slices of Pizza at the AP Biology Party, March 9, 1998, 7:00 p.m.–11:00 p.m.

You should be able to see that only title (e) is helpful and useful. It enables you to tell, by glancing at a calendar, whether or not you can attend the party, and it helps make that graph fall a little more steeply. The point we are driving at is that a *good* title is one that tells exactly what information the author is trying to present with the graph. Although brevity is desirable, it should not substitute for completeness and clarity.

Now that you are clear on titles, look at the graph in Figure A.5. Its title tells you that here is some potentially useful information. The graph suggests that, at least for 1998, there was an upper limit to the amount of time people could usefully spend in studying for an exam, and you might wonder, for example, how long you would have had to study to make a perfect score.

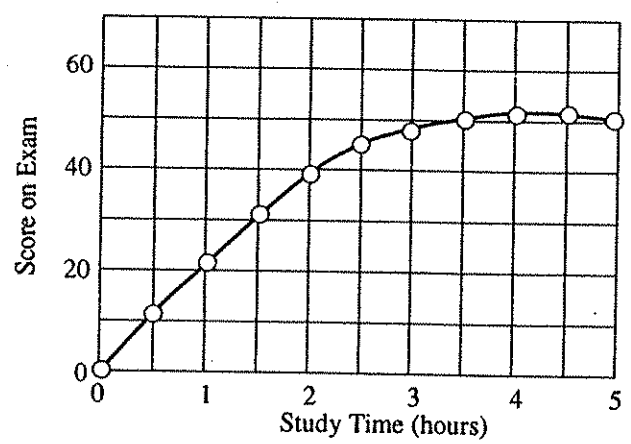
**Figure A.5: Relation Between Study Time and Score on a Biology Exam in 1998**



Unfortunately, however, you cannot tell, because the graph has no labels of numbers or units on the scales. Even though this graph has a descriptive and intriguing title, it is of no use to us at all without these very important parts. Obviously, before we can take full advantage of the information that the graph is trying to present, we need to have some additional details.

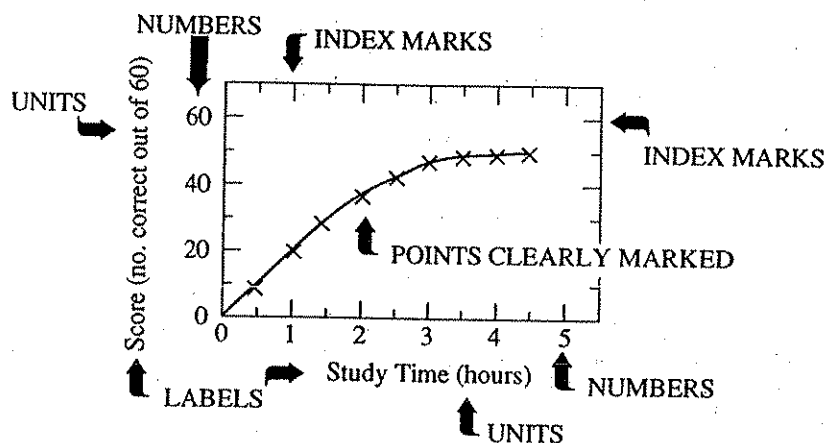
In Figure A.6 the additional information has been supplied, information that seems to make the graph more useful to us in preparing for the exam.

**Figure A.6: Relation Between Study Time and Score on a Biology Exam in 1998**



This additional information includes scales, or axes, that are carefully marked with numbers, and labels and units that are neatly presented. Obviously, one cannot label all the points along the axes; that would make the numbers crowd together and look sloppy. The units should be marked at intervals that correspond more or less to the intervals between the experimental points. The small marks, called **index marks**, can be drawn in if the experimental points are very widely spaced. Most elegantly, a **frame** is put around the whole graph, and index marks are placed all around. This makes it easy to lay a ruler across the graph when interpolating between the experimental points. The diagram in Figure A.7 summarizes some features of a good graph.

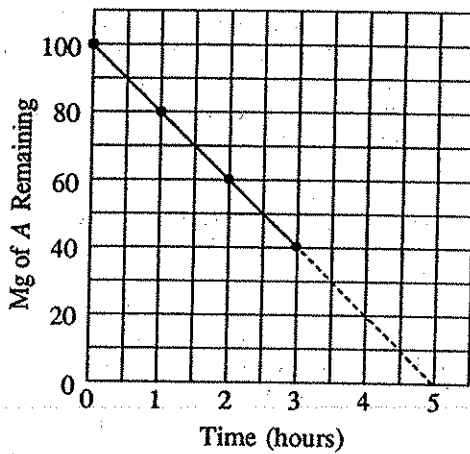
**Figure A.7: Relation Between Study Time and Score on a Biology Exam in 1998**



### Steepness or Slope of a Line Graph

Look at the graph in Figure A.8 for the disappearance of *A* in a chemical reaction. Such a graph, in which the amount of some quantity is shown on the vertical scale, or **ordinate**, with time shown on the horizontal scale, or **abscissa**, is frequently called a "progress graph" or "progressive curve," because it shows how some process progresses in time. This graph may also be called a "time course" for the process, because it shows the extent to which the process has occurred at different times.

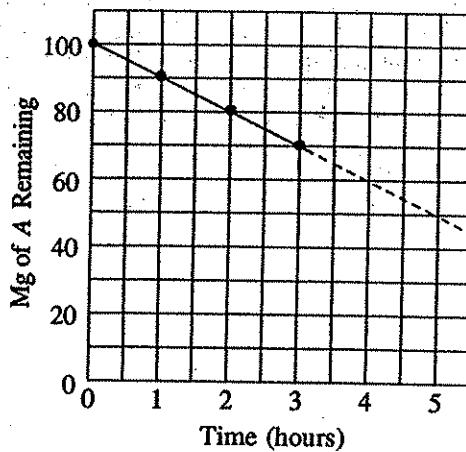
Figure A.8



Time Course of Disappearance of A in Process I

Let us call the process represented by the graph "Process I" and consider another reaction, "Process II," in which A is also consumed. Suppose that we start Process II also with 100 mg of A, and that after 1, 2, and 3 hours there are 90, 80, and 70 mg, respectively, left. The progress curve for Process II is displayed in Figure A.9.

Figure A.9

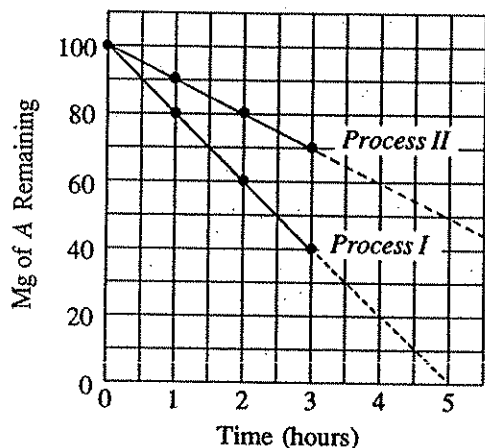


Time Course of Disappearance of A in Process II

Now, suppose we want to compare the graphs for the two processes. Because they have exactly the same scales, we can put both lines on the same graph, as shown in Figure A.10. Notice, however, that now in addition to the labels on the scales, we need labels on the two lines to distinguish between the two processes.

Look at the 1-hour mark on the time scale of the graph. Opposite this put an X on the line for Process I and a Y on the line for Process II. Then, opposite X on the ordinate you should be able to see that 80 mg of A are left in Process I; opposite Y you can see that 90 mg of A are left in Process II. Apparently, Process I has used up 20 mg of A and Process II has used up only 10 mg in the same amount of time. Obviously, Process I is faster, and the line graph for Process I is steeper than the graph for Process II.

Figure A.10



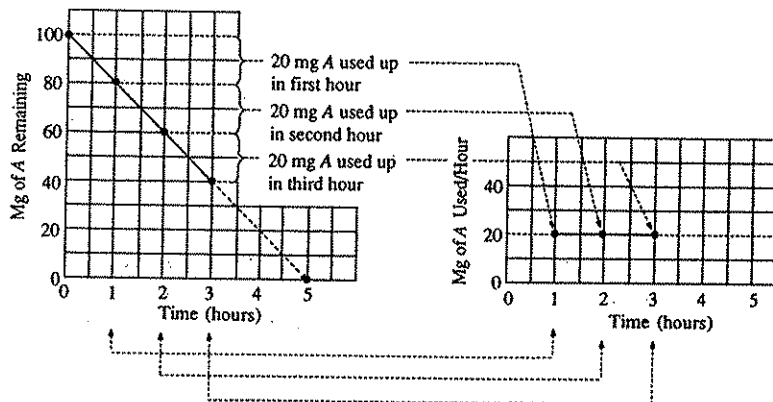
Time Course of Disappearance of A in Processes I and II

The rate for Process I is 20 mg A used/hr, while the rate for Process II is 10 mg A used/hr.

We have seen that a steeper line graph means a faster reaction when the progress curves for two reactions are plotted on the same scale. (Obviously, if the progress curves are plotted on different scales, we cannot compare the steepness of the line directly, but have to calculate what the slope would be if the two curves were plotted on the same scale.)

Suppose, now, that we make a new kind of graph, one that will show the steepness, or slope, of the progress curve. Because the slope of the progress curve is a measure of the speed of velocity, or rate of the reaction or process, such a graph is frequently called a "rate graph" or "rate curve." The diagram in Figure A.11 shows how a rate curve can be made for Process I.

Figure A.11

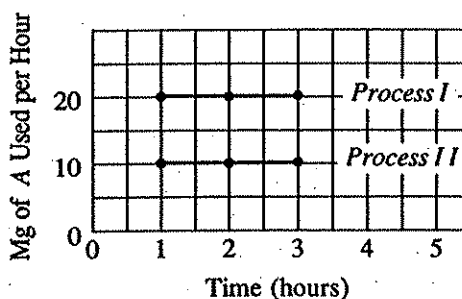


Notice that the time scale of this rate graph is exactly like the time scale of the progress curve from which it was derived, but that the ordinate is different. The ordinate of the progress curve shows milligrams of A remaining; the ordinate of the rate curve shows milligrams of A used per hour. Obviously, a rate graph must always show rate on one of its scales, and it is ordinarily the vertical one that is used. This is because the rate of a reaction or process is what mathematicians call a **dependent variable**. Time is the **independent variable** in this experiment; it is independent of changes in the dependent variable (the rate of reaction), and it is the variable that is shown on the horizontal axis. Regardless of whether the process is the increase in height or weight of a plant, or the using up or producing of something in a reaction, the rate graph for the process must always show *amount of something per unit time* on one of its axes. One very common type of rate graph is the one shown in Figure A.11, with a rate on the ordinate and the time on the abscissa. Other kinds of rate graphs may have temperature or molarity on the abscissa. The rate of growth of a plant, for example, depends on many factors that we might wish to vary, and so we can have as many different kinds of rate graphs for that process as there are independent variables.

Let us emphasize: a progress curve always shows amount of reaction on the vertical scale and time on the horizontal scale. The corresponding rate curve *may* show time or some other variable on the horizontal scale, but it *always* shows rate, or amount of reaction per unit time, on the vertical scale. This point is very important. When we look at a rate curve that has time on the horizontal scale, we must visualize the progress curve from which the rate curve was derived. When we look at a rate curve that has any other variable except time on the horizontal scale, we shall see that each point on that rate curve represents a separate progress curve.

In the same way as for Process I, a rate curve can be made for Process II. Plotted on the same graph, the two should look something like the diagram in Figure A.12.

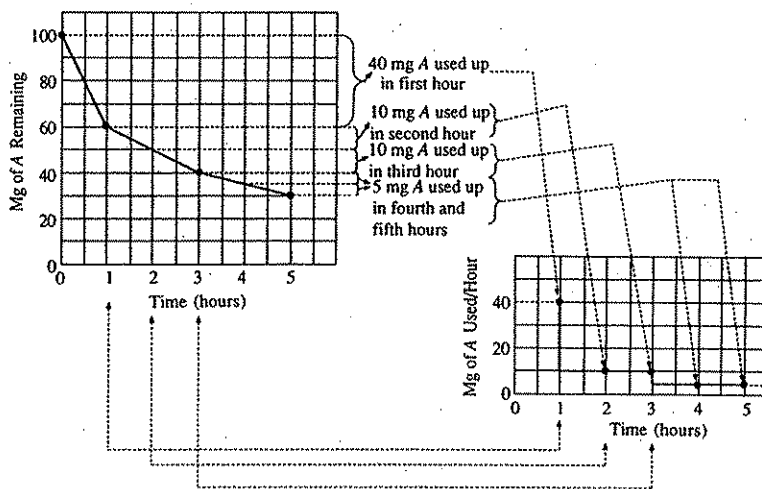
Figure A.12



There are two things to notice in this example. First, the curve for Process I lies higher than that for Process II. This is in accord with the facts as we have seen them, namely, that Process I is faster and so has a greater slope or higher value for the steepness. Second, notice that both curves are perfectly flat. Naturally, because the progress curves for the two processes were both perfectly straight lines, having everywhere the *same* slope, the rate of steepness graph must show exactly the same thing, that is, that the rate or steepness is everywhere the same.

On the other hand, consider the graph in Figure A.13, which represents the disappearance of A in yet another reaction, Process III.

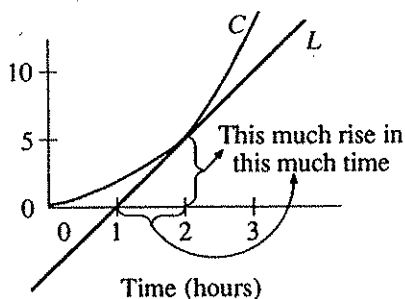
**Figure A.13: Time Course of Disappearance of A In Process III**



You can see that Process III differs from Processes I and II in that the progress curve for III is not a perfectly straight line. It is steepest at the beginning, becomes less steep after 1 hour, and again after 3 hours. Obviously, because the rate of the process is changing with time, the corresponding rate curve will *not* be perfectly flat. The rate has to start out high, then drop at 1 hour and at 3 hours, and you can see in the graph on the right that this is exactly what it does. In fact, the rate curve looks like steps, because whenever the slope of the progress curve decreases, the rate curve must show a drop to a lower value. Conversely, if the progress curve for a process should get steeper, as sometimes happens (the reaction goes faster after it gets "warmed up"), the rate curve must show a corresponding increase to a higher value.

Until now we have been able to read the steepness, or slope, of the progress curve directly from the scales of the graph, because the progress curves we have been studying were either perfectly straight lines or else made up of straight-line segments. In most real situations, however, we cannot do this, because the slope of the progress curve does not change sharply at a given time, but gradually, over a period of time. You probably remember how to measure the slope of a curved line, but let us review the process anyway. (See Figure A.14.)

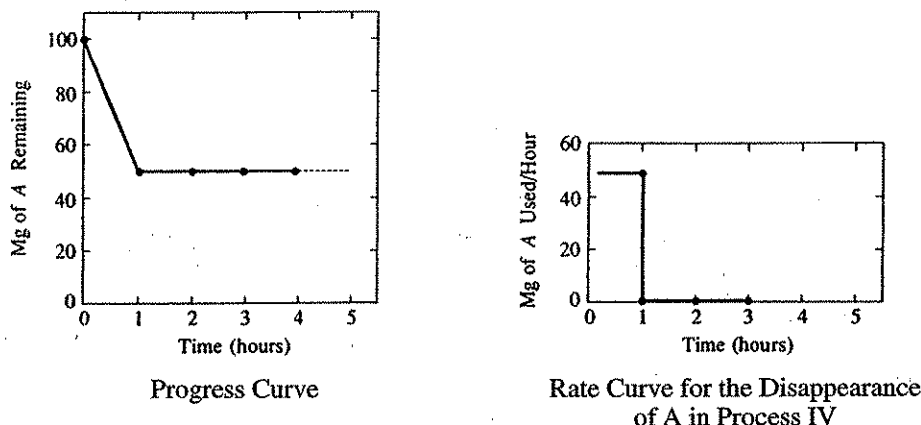
**Figure A.14**



Suppose we want to measure the slope, or steepness, of the curved line C at time 2 hours. We can see that the curve rises 5 units total in the 2 hours, so that the average slope is 2.5 units per hour. However, it is easy to see from the graph that this average is very misleading; the progress curve is almost flat at the beginning (i.e., has 0 slope) and then accelerates rapidly, so that the line curves upward. If we want to find the true slope at 2 hours, we must draw line L in such a way that L has the same slope as C at the 2-hour point. Then we can see that L rises about 5 units between 1 and 2 hours, just twice the average slope for the first 2 hours.

We have seen that a perfectly flat rate curve, like that for Process I or II, means that the corresponding progress curve is a perfectly straight line having the same slope at all points. Conversely, a progress curve that changes in slope, like that of Process III, will give a rate curve that looks like steps. You should be able to figure out that the "steps" on the rate curve will be sharp and square if the progress curve has an abrupt change in slope, and more rounded off if the progress curve changes slope gradually. In any case, in regions where the rate curve is perfectly flat it is clear that the progress curve must have constant steepness, or slope. However, if the progress curve itself gets perfectly flat, then that portion of the progress curve has 0 slope; in other words, the reaction has stopped. This kind of situation is pictured in Figure A.15 where the rate and progress curves for another reaction, call it Process IV, are shown.

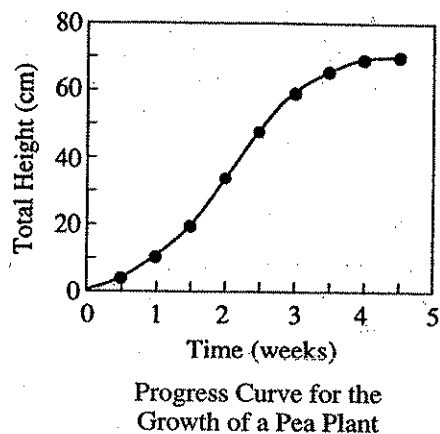
Figure A.15



In the progress curve on the left, we can see that after the first hour the reaction stopped. From the graph we can see that after 1 hour there were 50 mg of A remaining; after 2 hours there were still 50 mg remaining; and there are still 50 mg remaining even at 4 hours. Obviously, Process IV stopped when one-half of A had been used up. Now look at the rate curve on the right. It is perfectly flat for the first hour, because the slope of the progress curve was constant during that time. After the first hour the rate curve is also perfectly flat but it has dropped down to 0, indicating that although the progress curve has constant slope, the slope is actually 0. Obviously, flatness in a rate curve and flatness in a progress curve mean different things. Flatness in the progress curve for a reaction means that the reaction has stopped; flatness in the rate curve means that the reaction is going on at a constant rate. You can see, then, that we have to be able to glance at a graph and tell whether it is a rate curve or a progress curve in order to be able to interpret what the shape of the curve is trying to tell us.

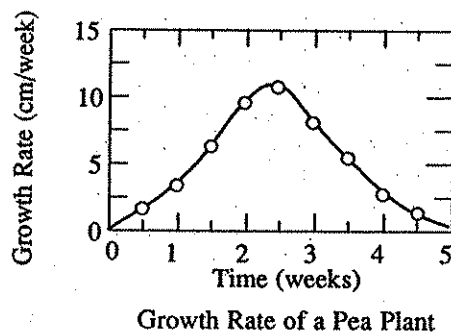
Now let us take one more example of this kind of rate curve. The graph in Figure A.16 shows the progress in the growth of a pea plant. First, we can see that the slope is not the same everywhere. In fact, there is an interval where the slope increases very gradually from 0. By 1 week or so the slope has reached its maximum value and is steady until about 3 weeks. Thereafter, the slope begins to decrease again, as the curve bends over, and eventually, at about 4.5 weeks, as the curve gets perfectly flat, the slope, or steepness, tends to be 0 again.

**Figure A.16**



Suppose, now, that we try to imagine what the rate curve for the growth of this pea plant will look like. If you read through the preceding paragraph, you will have a rough description of it. In fact, it will look like the graph in Figure A.17.

**Figure A.17**



Notice from the two graphs that where the steepness of the progress curve gets larger, the corresponding rate curve turns upward. Similarly, when the slope of the progress curve decreases again, the rate curve turns downward. A rate curve that is turning up means, therefore, that the process is speeding up; a flat rate curve means that the process is going at a constant rate; and a rate curve that is turning down means that the process is slowing down. When the rate curve hits the x-axis, it means that the reaction has stopped.

Probably 80 percent of the graphs you will encounter in biology are either rate curves or progress curves. You will have noticed from the preceding discussion that biologists tend to use the words "graph" and "curve" interchangeably. Technically, of course, the entire picture, including the abscissa, ordinate, labels, numbers, units, index marks, and title, together with the line graph portrayed, is the "graph," while the line graph itself is called the "curve." You will notice, too, that biologists call a line graph a "curve," even though the line itself may be perfectly straight.

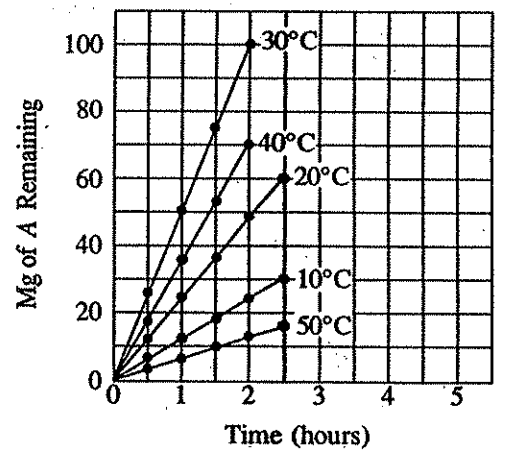
To summarize, remember that a progress curve is made from measurements at different times during the progress of a reaction that is continuous with time. A graph that shows how much or to what extent a reaction has occurred at different times is a progress or time-course curve. In contrast, a rate curve is a picture of the steepness of one or more progress curves, and any graph that has rate on one of its scales is a rate curve.

So far we have been considering only rate graphs that have time on the abscissa; we could call these **time-rate** curves. As we have seen, a time-rate curve can be made from any progress curve. Next, we are going to consider rate curves that do not have time as the abscissa. As you shall see, such curves are made by combining data from several progress curves, each representing the time course of the reaction under a different set of conditions.

### Other Kinds of Rate Graphs

Let us look at and try to analyze the graph in Figure A.18. Obviously, it is a progress curve, because it shows an amount of something on the ordinate and time on the abscissa. There are several different curves all plotted on the same graph, and each is labeled with a different temperature. The title indicates that this graph is trying to tell us how Process I behaves at different temperatures.

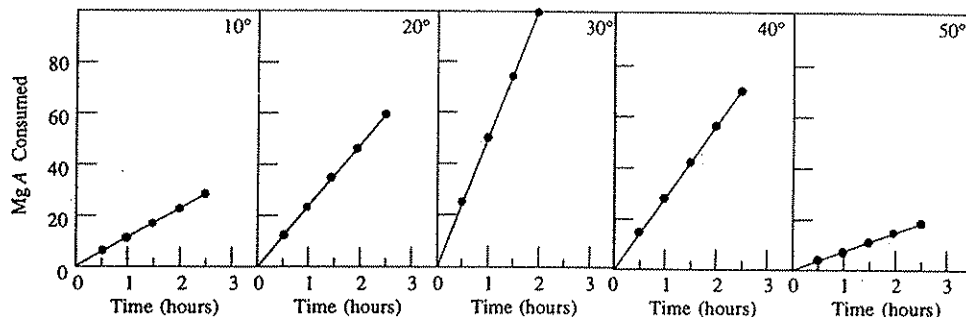
Figure A.18



Temperature Dependence of Process I

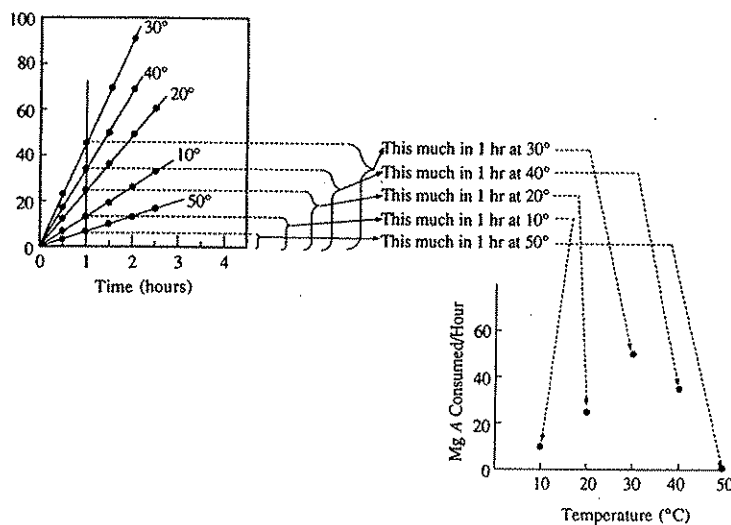
Before we try to construct the rate curve for this graph, we should try to imagine how this experiment was carried out. It seems clear that the experiment must have started with several different batches of A and that each reaction mixture was kept at a different temperature. Then, every half-hour, the amount of A remaining was measured and the amount consumed was calculated. The results might have been plotted in five separate progress curves, as shown in Figure A.19.

Figure A.19



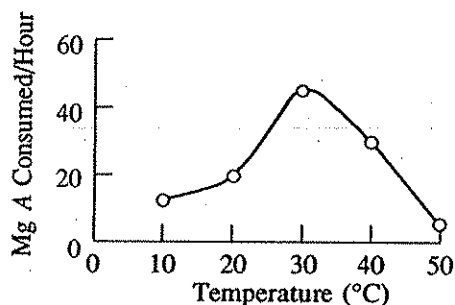
When all these progress curves are plotted on the same graph, as was done in Figure A.18, we have what is called a "family" of curves. If we look at the slopes of the various members of the family of curves for Process I, we see that the steepest slope does not correspond to the highest temperature. In fact, the curve for 30° is the steepest, whereas the curve for 50° is the least steep; the curve for 10°, the lowest temperature, has an intermediate slope. By analyzing and comparing the slopes of the family of curves in this way we can get a reasonably good notion of the effect of temperature on Process I, but this effect could be shown much more clearly in a rate graph that has temperature as the abscissa. Such a graph would show us at a glance how the rate varies with temperature and, of course, would be preferable, as the whole point in making a graph is to present information simply and clearly. The diagram in Figure A.20 shows how a rate-temperature graph would be constructed from this family of curves for Process I.

Figure A.20



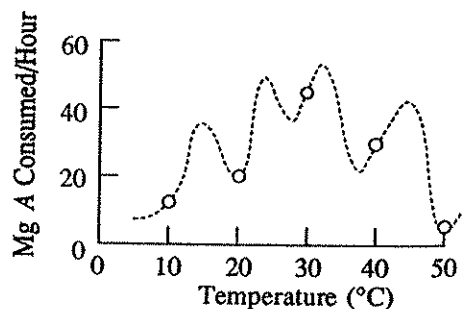
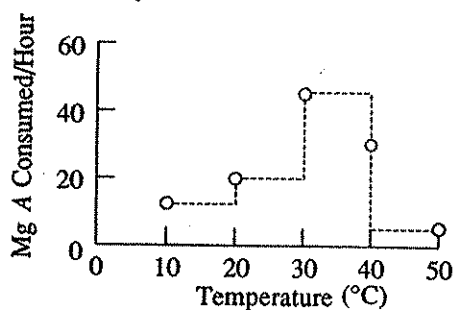
Having found, as shown in Figure A.20, the five points for our rate graph, we are faced with the question of whether or not it is legitimate to connect these points with a smooth line. We recognize, of course, that both temperature and rate are continuous processes. Between any two given temperatures or rates there are an infinite number of temperatures or rates. The question here, however, is the following: If we do draw a smooth line through our five points, will that line pass through the infinite number of other rates that we could have measured if we had chosen some other temperature? Let us go ahead and draw the line, as shown in Figure A.21.

Figure A.21



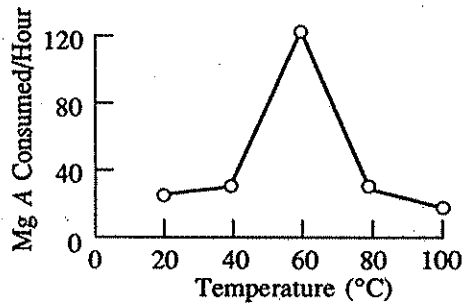
As we have drawn it, the curve indicates that the rate at 29° and at 31° would be slightly lower than at 30°, and this may not be true. In order to determine the true shape of the curve in the region of the maximum rate we would have to make progress curves at smaller temperature intervals, say, every two degrees. However, it is extremely unlikely that the true shape of the curve is anything like the two possibilities shown on the diagrams in Figure A.22. All our experience tells us that if a reaction depends on temperature, then that dependence will be a smooth curve, without sharp bends. In fact, if in an experiment we *should* observe behavior of the type shown in Figure A.22, we would immediately begin to suspect that something is wrong with our thermostat! Thus, although it may be that the shape of the rate-temperature curve for Process I is somewhat different from the way we drew it in Figure A.21, we can be reasonably certain that it is not radically different.

Figure A.22



In addition, we may also tend to be suspicious of a graph if we see a sharp peak, unless the experimental points were taken very close together. For example, common sense would tell us to be careful about accepting the rate curve shown in Figure A.23.

Figure A.23



Obviously, most of the shape is given to the profile by the one measurement at 60°. In biology, as in everything else, mistakes can be made, so the experimenter would have to check the validity of that measurement very carefully. The easiest way to do that would be to make more measurements slightly above and slightly below 60° to see whether these would fall on the line the experimenter has drawn. Alternatively, the experimenter could play it safe and draw only a bar graph for these spaced temperatures. Another useful dodge would be to connect the points with a smooth but broken line rather than a continuous line. As always, the broken line would suggest the tentative and provisional nature of the curve as drawn.

