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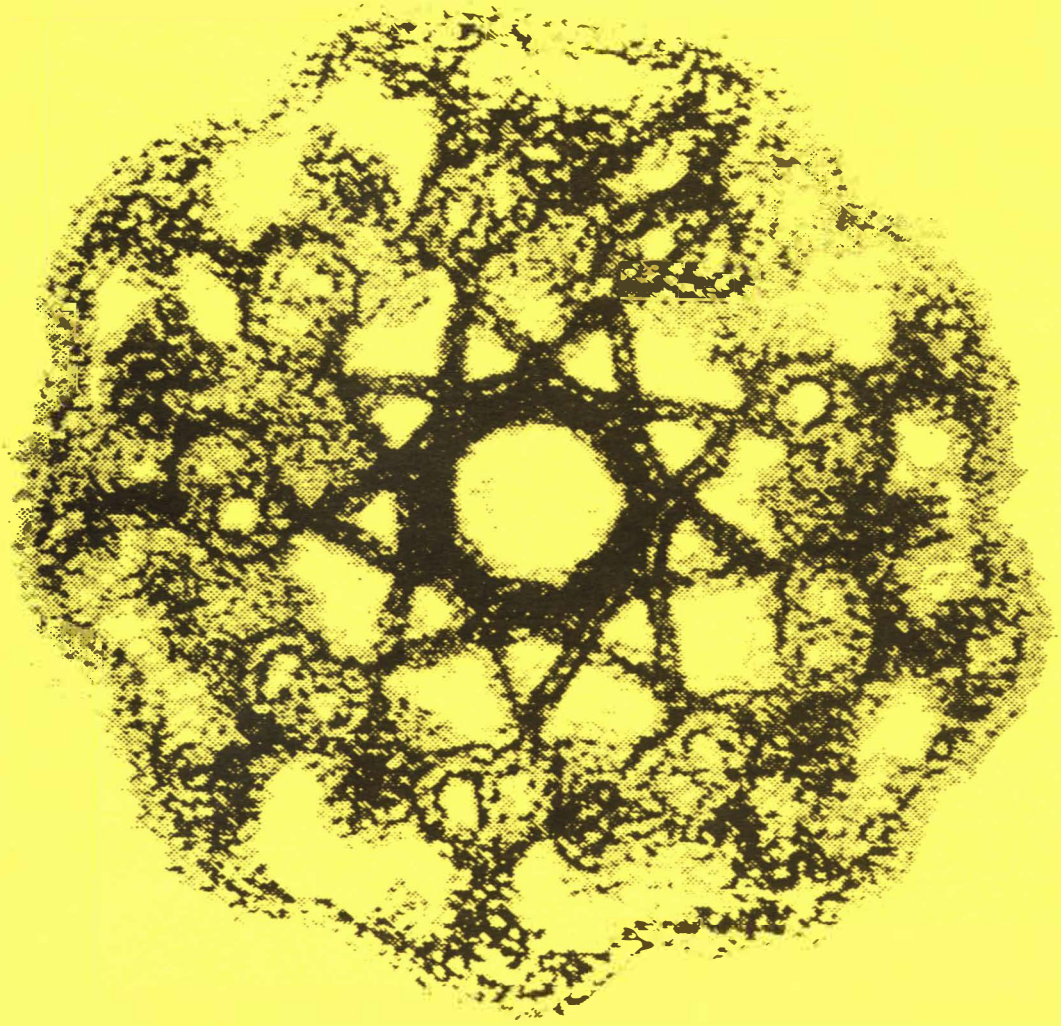

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Electron micrograph of a cross section through the transition zone of a Chlamydomonas flagellar axoneme. Note the stellate array of centrin-based fibers that connect the central cylinder to the microtubule doublets. x990,000. For more details see: "Centrin-mediated Microtubule Severing during Flagellar Excision in *Chlamydomonas reinhardtii*" by Mary A. Sanders and Jeffrey L. Salisbury, Journal of Cell Biology 108:1751-1790 (May 1989).

Photograph courtesy of Mark Sanders and Jeffrey Salisbury.

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Hardware

Bioscene *is the official publication of the* ***Association of Midwestern College Biology Teachers***

USING RANDOM NUMBERS IN BIOLOGY

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INTRODUCTION

One of my goals as a college teacher is to promote biology as inquiry and provide opportunities for students to develop problem-solving skills. Random numbers are useful tools for implementing this approach to education. This paper reviews the techniques used to generate various distributions of random numbers, and suggests several classroom applications. The level is introductory, and assumes only a modest understanding of BASIC and spreadsheet operation.

A random number is unpredictable; that is, chance determines the value. Computers and calculators generate pseudorandom numbers, which are sequences of numbers that pass the statistical tests for randomness, although the numbers are calculated with various mathematical formulas (Pierce 1988, Press et al. 1986). In this paper I use the terms pseudorandom and random interchangeably. Monte Carlo techniques use random numbers to simulate and solve problems that have some mathematical basis. The name comes from Monte Carlo, a gambling resort in southeastern France.

GENERATING RANDOM NUMBERS

Most calculators and computer languages, such as BASIC and Pascal, generate random numbers in the range greater than or equal to zero but less than one ($0 \le N < 1$). In most versions of BASIC, for example, RND(S) is the random number function. The operator S determines the nature of the result: $S > 0$ calls the next random number ($0 \le N < 1$). The magnitude is unimportant, and, in most BASICS, may be omitted; $S = 0$ calls the previous random number; $S < 0$ starts a new, repeatable sequence. Different negative values for S produce different sequences of psuedorandom numbers, but identical values result in the same sequence. Here is an example:

```
10 R1 = RND (-2): REM  -S starts new sequence
20 GOSUB 100
30 R1=RND(-6): REM New -S starts new sequence
40 GOSUB 100
50 R1 = RND (-2): REM  Repeats first series
60 GOSUB 100
70 GOTO 200
100 FOR I = 1 TO 4
110 PRINT RND (1): REM  Any +S calls next
     number
115 NEXT I
118 PRINT
130 RETURN
200 END

   .6624333  .7354596  .1928332  .4788546
   .4124333  .4854596  .9428332  .2288546
   .6624333  .7354596  .1928332  .4788546
```

Most versions of BASIC have a RANDOMIZE statement that we use to seed the random number generator. It is often combined with the TIMER function to produce a unique seed. The TIMER function records the elapsed times since the last system reset.

The random number generator in Applesoft, the BASIC in the Apple II family of computers, has two flaws. The cycle is short so that the sequence of random numbers begins to repeat after as few as 2000 calls (Lingwood 1981). The short cycle should not be a problem for applications requiring only a few hundred random numbers.

Gleason (1988) shows that the choice of seed values affects the length of the cycle. The second problem is more serious, but easily fixed. The random number generator is not correctly seeded on start up, so the same sequence of pseudorandom numbers appears each time an Apple II is turned on. Applesoft lacks a RANDOMIZE statement but we can use memory locations 78 and 79 produce a random seed (Sparks 1989):

```
10 PRINT "PRESS ANY KEY ";: GET A$: PRINT
20 S = PEEK (78) + PEEK (79) *256
30 R1 = RND(-S)
```

Locations 78 and 79 change rapidly while waiting for a key press, producing a value for S between 1 and 65535.

Most spreadsheets and some database programs generate pseudorandom numbers. The spreadsheet function @RAND (or its equivalent) generates a pseudorandom number between 0 and 1 ($0 \leq N < 1$). Recalculation produces a new random number. I know of no spreadsheet that permits modification of the seed.

TESTING RANDOMNESS

The statistical methods used to test for randomness include the Chi Square test, and a comparison of observed frequencies with the frequencies expected from the Poisson distribution (Gleason 1988, Spain 1982: 252). Dowdy (1986: 215) gives a BASIC program listing which examines the properties of computer-generated random numbers, including the relative frequencies of odd and even digits and the relative frequency of digits 0 through 9. We expect, of course, that odd and even digits are equally represented, and each digit 0 through 9 appears 0.1 of the time.

A qualitative, but entertaining test of randomness involves generating random X, Y coordinates and plotting these on the graphics screen. Dots gradually fill the screen, but on the Apple II new dots stop appearing after several minutes, showing that the random number generator has entered a repeating sequence. This does not happen in MS DOS versions of BASIC, or with languages which bypass the Applesoft generator.

For an Apple II the code is:

```
10 PRINT "PRESS ANY KEY TO START OR
   STOP ";: GET A$: PRINT
20 R1 = RND(-(PEEK(78 )+PEEK(79)*256))
30 HGR2: HCOLOR=3
40 HPLOT RND(1)*280, RND(1)*192
50 GOTO 40
```

The MS DOS version of the program is:

```
10 RANDOMIZE TIMER
20 SCREEN 2
30 PSET (RND(1)*640, RND(1)*200)
40 GOTO 30
```

DISTRIBUTIONS OF RANDOM NUMBERS

Computers and calculators produce uniform distributions of pseudorandom numbers between 0 and 1 ($0 \leq N < 1$). For most applications we need to convert those numbers to fit a particular distribution. The formulas below are BASIC statements with descriptive variable names.

Uniform Distribution with a Specified Range

The formula is:

```
10 R1 = RND(1) * (HIGH - LOW) + LOW
```

If we set LOW and HIGH to 25 and 100, respectively, R1 equals a floating point number 25 - 100, inclusive.

Uniform Distribution of Integers with a Specified Range

The formula is:

```
10 R1=INT(RND(1)*((HIGH-LOW)+1))+LOW
```

INT is the BASIC statement which takes the integer value of the argument.

Normal Distribution with Specified Mean and Standard Deviation

The mean and standard deviation of a series of numbers generated by this method (Spain 1982: 272) approximates

the specified values for the mean and standard deviation:

```
10 R1 = RND(1)
20 R2 = RND(1)
30 R3 = 0.603 * LN(R1/(1-R2))
40 R3 = R3 * STANDARD_DEVIATION + MEAN
```

Scheuermann (1989) and Duhrkopf (1990) present alternative formulas to produce random normal deviates.

Binomial Distributions

Binomial distributions result from repeated, independent trials with two possible outcomes and fixed probabilities. Binomial probabilities find widespread use in problems and simulations in genetics and evolution. The general technique involves a programming loop set to the size of the class. Random numbers less than or equal to the probability of one class are tallied. The frequency of the other class is found by subtraction.

```
10 CLASS_1 = 0
20 FOR COUNT = 1 TO
   SAMPLE_SIZE
30 IF RND(1) < = PROB_
   OF_SUCCESS THEN
   CLASS_1 = CLASS_1+1
40 NEXT COUNT
50 CLASS_2=SAMPLE
   _SIZE - CLASS_1
```

Numbers in a Series with No Repeats

The general procedure is to store the first random number in the first element of an array. Successive random numbers are compared to existing numbers stored in the array. If the number already exists in the array it is rejected; if not it is added to the next element in the array. This method is quite fast for short lists of numbers.

Nolan (1988) describes a different method, which is interesting because it resembles some mathematical algorithms used to generate pseudorandom numbers. The following routine generates a random list of integers from 0 to HIGH - 1, where HIGH is a power of 2:

```
10 INPUT "HIGH VALUE EQUAL TO A POWER
   OF 2—> "; HIGH
20 MULTIPLIER = 13
30 RANDOMIZE TIMER
40 SEED = INT(RND(1) * HIGH + 1)
50 PRINT SEED: PRINT : PRINT
60 FOR COUNT = 1 TO HIGH
70 SEED = (MULTIPLIER * SEED+1) - HIGH*
INT((MULTIPLIER*SEED+1) / HIGH)80 R = SEED
90 PRINT R,
100 NEXT COUNT
```

Table 1. Spreadsheet template for various distributions of random numbers.

	A	B	C	D	E	F	G	H	I	J	K
1											
2		LOW->	0		LOW->	0		MEAN->	10	PROB->	0.3
3		HIGH->	7		HIGH->	7		SD->	0.1		
4			UNIF		UNIF	INTG			NORMAL		BINOM
5											
6											
7			1.730			3			10.04		0
8			4.632			2			9.861		0
9			5.813			1			10.19		0
10			6.108			3			10.01		0
11			6.402			7			9.772		0
12			5.137			1			9.997		0
13			2.636			1			10.00		0
14			0.604			7			9.992		0
15			1.171			7			9.994		1
16			5.316			7			9.974		1
17											
18											
19		MN ->	5.316		MN->	7		MN->	9.974	PROP->	0.2
20		SD ->	2.084		SD->	2.624		SD->	0.105		

Formulas:
Cells B7-B16 = @RAND*(B3-B2)+B2
Cells E7-E16 = @INT(@RAND*(E3-E2)+E2+1
Cells H7-H16 = 0.603*@LN(@RAND/(1-@RAND))*H3+H2
Cells K6-K17 = @IF(@RAND(<=K2,1,0)
Cells B19,E19,H19=@AVG(_6.._17)Cells B20,E20, H20=@STD (_6.._17)
Cell K 19 = @SUM(K6..K17)/@COUNT(K6..K17)
(Underscore (_) = respective column)

You can duplicate most random number routines on a spreadsheet (Table 1). The numbers under "UNIF" (column B) represent random floating point numbers in the range 0 through 7. Cells B7 through B16 contain the formula @RAND*(B3-B2)+B2. The numbers under "UNIF INTG" (column E) are uniform random integers in the range 0 through 7. Cells E7 though E16 contain the formula @INT(@RAND*(E3-E2)+E2+1. The numbers under "NORMAL" (column H) are random normal deviates with a mean and standard deviation of approximately 10 and 0.1, respectively. Cells H7 through H16 contain the formula 0.603*@LN(@RAND/(1-@RAND))*H3+H2 Finally, the numbers under "PROB" (column K) are binomial probabilities. Cells K7 through K16 contain the formula @IF (@RAND (<=K2,1,0). Each cell represents a loop in the BASIC routine described above. Cell K19 contains the formula @SUM(K6..K17)/@COUNT (K6..K17). This gives the proportion for the class size of 10. The formula references blank cells above and below the numbers in column K. Blank cells have no effect on the calculation, and this makes it easier to alter the template by inserting rows, deleting rows, and sorting.

Through appropriate use of absolute and relative cell references, and the copy function of spreadsheets, we can easily expand and contract this template. Recalculation produces new random numbers. We can change the distribution of our random numbers by altering the low, high, mean, standard deviation, and probability values in rows 2 and 3.

APPLICATIONS

This section explores classroom applications for random numbers. You can follow the literature citations for many additional examples.

Statistical Problems

The routines for generating normal and other distributions are ideal for preparing problems for Chi Square, Analysis of Variance, and other statistical tests. A spreadsheet is especially suitable because the results can be printed to a text file, and loaded into a word processor for additional editing.

At least one company (Oakleaf Systems in Decorah, Iowa) has expanded this idea, and markets a program to produce individualized problems. Cauchon (1982) illustrates how reproducible sequences of random numbers allow students or instructors to check answers to tutorials and drill and practice programs.

Deterministic vs. Stochastic Models of Population Growth

Students often have trouble understanding the effect of age-specific fecundity and mortality on population growth. This example, modified from Caughley (1974), Cody (1977a, 1977b), and Krebs (1972: 204), overcomes that problem, and, also, introduces students to the ideas of deterministic and stochastic models.

We begin with a deterministic model representing the growth (or decline) of a hypothetical organism with asexual reproduction, which dies after 2 years of age, and produces offspring at 1 and 2 years. A deterministic model is one that produces an exact outcome for a given set of inputs (rate of increase, initial population size, and time). Table 2 shows the life history of our example organism. Readers unfamiliar with life and fecundity tables should consult a good ecology text or refer to Table 3, which summarizes the calculations.

To see how this population grows, we start with a few individuals and follow this population through several generations by applying the age specific rates of survival and reproduction from the fecundity table (Table 2). Table 4 is a partially worked out example commencing with a popula-

Table 2. Fecundity table for a hypothetical, asexually reproducing organism used in the model of population growth.

x	f_x	l_x	d_x	q_x	m_x	V_x	xV_x
0	6	1.00	0.50	0.50	0	0	0
1	3	0.50	0.17	0.33	1.0	0.500	0.500
2	2	0.33	0.33	1.0	3.5	1.155	2.310
3	0	0					
R_0 =		1.655				2.810	

tion of 3 newborns (0 year age class) and 3 yearlings (1 year age class). The rate of mortality is 0.5 and 0.33 for the 0 and 1 year age classes, respectively. Thus, one half of those in the 0 age class survive and become yearlings.

Similarly, 0.67 (1 - 0.33) of yearlings survive and become 2 year olds. After calculating survivorship, we find the total fecundity. Animals 0 years old do not reproduce. Each 1 year old produces 1 offspring and each 2 year old produces 3.5 offspring. The total reproductive output becomes the new 0 age class.

Once these calculations are carried out for 10 - 20 generations, it is interesting to compare the pattern of growth with the mathematical representation of intrinsic growth:

$$N_t = N_0 e^{rt}$$

where N_t is population size at time t, N_0 is initial population size, e is base of natural logs, r is the instantaneous rate of increase, t is time (years in this example). There are two ways to estimate r, the rate of increase. The survival fecundity rate of increase, symbolized rs, is calculated from the fecundity table as follows:

1. Calculate the mean generation time.
$G = \Sigma(xV_x)/R_0 = 2.81/1.655 = 1.698$

The xV_x is found by multiplying each V_x by each x (the age) and adding the results for all age classes.

2. Find r_s.

$r_s = (\ln(R_0))/G = 0.297$ per individual per year (ln mean natural logarithm).

3. Refine the estimate by iterative solution to the equation:

$$\Sigma(e^{-rx})(l_x m_x) = 1$$

The best estimate of r_s after several interations is 0.306. Caughley (1977: 110) and Krebs (1972: 171) provide a complete discussion of these calculations.

The second method of estimating r is to fit the exponential equation of the form $y = ae^{nx}$ to the population growth data, where x equals generations (time) and y is total population size. We do this by calculating a least squares regression equation using natural logs (ln) of the total population size as the y data. The actual y intercept equals e^a. The slope (n) is reported in the correct units, and is called the observed rate of increase. Many spreadsheets have a regression option, and the analysis can be completed in a few seconds.

The next step is to convert this exercise into a stochastic model. We do that

Table 3. Calculation of life history statistics in life and fecundity tables.

Column & meaning	Symbol	Calculation
Age	x	Use appropriate grouping
Observed no. alive	f_x	Original data
Probability at birth of living to age x	l_x	May use 1 or 1000 as starting cohort (x = 0) $l_x=(f_x)(l_{x-1})/(f_{x-1})$
Number dying in interval x to x+1	d_x	$d_x=l_x - l_{x+1}$
Mortality rate in	q_x	$q_x=d_x / l_x$ interval x to x+1
Fecundity: Offspring per female of age x	m_x	Original data
$(l_x)(m_x)$	V_x	$(l_x)(m_x)$
Net reproductive rate	R_0	$R_0 = \Sigma V_x$ (multipication rate per generation as finite rate)
Summation of xV_x	xV_x	ΣxV_x (used to calculate mean generation time and r)

by simulating birth and death with random numbers generated according to the probability of those events. The survivorship for the 0 age class is 0.5, meaning that, on the average, 50 percent of animals in the new born age class live to become yearlings. In the deterministic model the 0.5 was fixed; in this stochastic model, we can represent it as a mean by using random numbers. We could flip a coin to decide if a given individual survives, or we can set up a spreadsheet template with the formula @IF ((@RAND>0.5) ,"Live" ,"Die"). If the 0 age class consists of 5 individuals, the sum of five recalculations or the sum five cells containing the formula gives the number of survivors which enter the yearling age class. Similarly, 0.67 of individuals 1 year old survive to become 2 year olds. The spreadsheet formula becomes: @IF((@RAND>0.67), "Live","Die").

Age specific fecundity is assigned using the formulas @INT(@RAND*3), for yearlings (m_1) and @INT(@RAND*6+1) for two year olds (m_2). This means that 1 year olds may have 0, 1, or 2 offspring with equal probability. Two year olds may have from 1 to 6 offspring with equal probability. The means remain 1 and 3.5 for the age classes 1 and 2, respectively. Table 5 contains 10 replications of each of the formulas. If your classroom has only one computer available, print the spreadsheets in advance, recalculating between each sheet. Although Table 5 contains all formulas, I recommend separate sheets for each age class's survivorship and fecundity.

Each trial of the stochastic simulation produces unique results; however, the mean population size of several trial approximates the outcome of the deterministic model. The standard deviations are large because different litter sizes occur with equal probability. A more realistic approach would be to assign higher probabilities to some litter sizes than others.

My students invariably develop a better understanding of the mathematics of exponential growth after working through the deterministic model, and begin probe questions relating to age at first reproduction, the effect of age distribution and so on. You can convert the entire operation to a spreadsheet, where it resembles the Leslie matrix.

Table 4. Partially completed worksheet for deterministic population growth. Total population size (N_t) is rounded to whole units. See text for explanation.

Age (x)	Generation (t)							
	0	1	2	3	4	5	6	7
	m_1->	1.5	4.3	3.9	7.1	8.1	12.5	
	m_2->	7.0	3.5	10.2	9.1	16.8	18.9	
0	3	8.5	7.8	14.1	16.2	24.9	31.4	
	x	x	x	x	x			
	0.5	0.5	0.5	0.5	0.5			etc.
1	3	1.5	4.3	3.9	7.1	8.1	12.5	
	x	x	x	x	x			
	0.67	0.67	0.67	0.67	0.67			
2	0	2.0	1.0	2.9	2.6	4.8	5.4	
Nt	6	12	13	21	26	38	49	

Additional Examples and Sources

Spain (1982) devotes at least two chapters to simulations and exercises involving random numbers. Although many of his discussions are advanced, he does include several introductory examples. Starfield and Bleloch (1986) present several probability models for those interested in wildlife ecology. Dowdy (1986) is an excellent source for purely statistical applications. Crandall (1984) suggests several short random number problems, including:

1. Use random numbers to simulate the male and female births in 100,000 offspring. Modify the program to locate the longest unbroken string of females. Modify the program still further to find experimentally

the number of families of 5 (2 parents, 3 children) out of 200,000 families which have three daughters and no sons.

2. Model simplified particle diffusion, assuming that a particle starts at coordinates 0,0 and moves a set distance north, south, east, or west with equal probability (0.25). After several moves, calculate the distance from the starting point and compare the results with the expected value, equal to $\sqrt{(\pi/4)}$.

Table 5. Spreadsheet template to generate survivorship and fecundity data for the stochastic model of population growth.

Formulas:
Column under l1 = @IF((@RAND>0.5),"Live","Die")
Column under l2 = @IF((@RAND>0.67),"Live","Die")
Column under m1 = @INT(@RAND*3)
Column under m2 = @INT(@RAND*6+1)

Trial	l1	l2	m1	m2	Trial	l1	l2	m1	m2
1	Live	Live	0	4	6	Die	Live	1	1
2	Live	Die	2	6	7	Die	Live	0	3
3	Die	Live	0	5	8	Live	Die	1	5
4	Die	Live	0	4	9	Die	Live	1	1
5	Die	Live	2	1	10	Live	Live	0	4

CONCLUSION

Critics argue that computer simulations, especially those used in classrooms, are simplistic and fail to represent complex biological phenomena. Indeed, some simulations are less realistic than we might want, but that does not diminish their value as a teaching tool. Random numbers provide a way to improve the realism of simulations and models by introducing some of the variation which characterizes most biological phenomena.

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Communities of the Biological Crossroads: An Extraordinary Outdoor Classroom

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INTRODUCTION

In the north central part of Nebraska lies a very unusual stretch of the Niobrara River valley. The very unique geological, climatological, and biological circumstances that occurred there have combined to make this thirty-mile segment the "biological crossroads" of North America. Incredible diversity occurs in that thirty miles of river valley, where east meets west and north meets south. These ecologically diverse plants with their associated animals, meet, mix, and hybridize in this thirty miles. In all, 160 species find the limits of their distribution in this short stretch of the Niobrara (1). The seven major plant associations which occur here, the unique geology and paleontology of the valley, and the scenic beauty and remoteness of the area combine to provide an invaluable natural laboratory and a superlative "outdoor classroom" for a variety of field studies.

GEOLOGIC HISTORY

With the Rocky Mountain uplift at the close of the "age of dinosaurs", Nebraska ceased to be the bottom of a shallow Cretaceous sea. Initially covered with a grayish sediment which became Pierre shale, the face of the region was ultimately formed and sculptured through extensive erosive events and outflows from the uplifted mountains. Deposition of the Tertiary units of this region began in Oligocene times with the White River group, and continued with the Miocene Rosebud Formation and the Pliocene Ogallala, Ash Hollow, and Long Pine Formations. The Valentine sand of late Miocene/early Pliocene is a unit within the Ogallala Formation. These formations were layed down through the depositional activity of streams flowing eastward from the newly emerged Rocky Mountains (4). Although the initial uplift of the Rockies occurred near the end of the Cretaceous Period, pulses of this uplift continued through the Tertiary. In addition, the Chadron Arch of western Nebraska was uplifted during this time, and would have contributed to some of this deposition (5).

In early to mid Pleistocene, three major river systems became established as outwash streams, and ran eastward across the sloping face of Nebraska: the Arikaree/Republican in the south; the Platte system in the center; and the Niobrara in the north. The Niobrara rises in the high plains of east central Wyoming and snakes its way over 300 miles eastward, paralleling the So. Dakota-Nebraska border until it augments the Missouri River about 100 miles west of Sioux City, Iowa. For much of its several million year life, the Niobrara had meandered to and fro across a seven to eleven mile wide flood plain. Then recently, geologically speaking, it began its rapid downward cutting action near the present north edge, quickly creating a gorge about 400 feet deep and half a mile wide. The river sliced through a series of sedimentary formations in its descent—the Long Pine aggregate, the Ash Hollow concretion, the Valentine sand, the Rosebud siltstone, and into the Pierre shale, which makes up the riverbottom east of the Norden Bridge. In its meandering, on what is now the south side of the valley, the river removed the Ash Hollow formation during the Wisconsin

glaciation in late Pleistocene, and exposed the Valentine sand to wind and water action. The Valley of the Nirobrara at that time would have been broad and shallow, and centered somewhat south of its present location (4). This was associated with the four major glaciations, and their attendant climatic fluctuations during the Pleistocene period. In addition, the largely aeolian sculpturing of the Nebraska Sandhills was being carried out and subsequently completed. The Resistant Ash Hollow formation is still in place north of the river and extends into South Dakota as the Crookston Table. Further from the River, the Long Pine units form the Springview Table. In general, events of the late Pleistocene were responsible for the landforms now present (5). Thus was laid the groundwork for the evolution and development of seven different major plant communities in this short stretch of the Niobrara valley, centered on the Norden Bridge, which lies on the north-south access road between Norden, 10 miles to the north, and Johnstown, 16 miles to the south.

ECOLOGICAL HISTORY

The plant associations of this Niobrara valley changed time and time again, influenced and dictated by the erosion and outflow events, the advance and retreat of the glaciers, and the general climatological fluctuations associated with both. The seven present plant communities are the Eastern deciduous forest, the Rocky Mtn. pine forest, the Northern boreal forest, the tall-grass prairie, the upland mixed-grass prairie, the Pleistocene terrace mixed-grass prairies, and the Sandhills transitional mixed prairie (1).

EASTERN DECIDUOUS FOREST

During an extended period of moderate precipitation during the retreat and melting of the last glacier, an extension of the eastern deciduous forest developed over much of the area, especially on the protected north-facing south slope of the valley. Subsequently, warming and semi-arid conditions set in, and conditions necessary for the forest's survival retreated far to the east, into central Iowa, leaving remnant stands along the Missouri River and in the Niobrara valley.

The major woody plants present in this community can be seen in Table 1.

ROCKY MOUNTAIN PINE FOREST

In the cold, hibernal climate associated with the proximity of the last glacier, prior to and concurrent with the development of the eastern deciduous forest, the Rocky Mountain forest community invaded eastward in the valley, occupying especially the steep south-facing bluffs on the north side of the Niobrara. With the post-glacial warming trend, conditions suitable for this community receded westward to the Pine Ridge and Black Hills areas, and to the Rocky Mountains of Wyoming, leaving a remnant community behind.

The major woody plants present in this community can be seen in Table 1.

NORTHERN BOREAL FOREST

A unique and interesting plant association developed in the Niobrara Valley under a set of very stringent conditions. With the removal of the Ash Hollow concretion and the exposure of the Valentine sand, precipitation falling on the sand percolated downward to the impervious Rosebud siltstone beneath. Coincidentally, the siltstone along the south side of the valley was tilted slightly toward the river. Water accumulating on the siltstone flowed slowly toward the river, emerging as springs, seeps, and waterfalls. Some streams thus created fell directly into the river, while others flowed out and across the floodplain. Over time, these spring-fed streams eroded downward through the pink siltstone and created "springbranch" canyons, which have a unique cool/damp microclimate (1). These canyons developed simultaneously with the glacial retreat, during which time a northern boreal forest community was prevalent in the valley. Conditions suitable for the birch forest community moved north with the retreating glacier, leaving only the remnants in the springbranch canyons along the south side of the Niobrara

River. Today, the nearest approach of the northern boreal forest community lies along the No. Dakota-Canada border.

The major woody plants, trees, shrubs, vines of this community are can bee seen in Table 1. A rich variety of aquatic vegetation, including liverworts, mosses, club-mosses, ferns, stinging nettles, sedges, northern grasses, and spotted touch-me-not occur around the seeps and springs and along the water's edge in these canyons. Ponderosa pine and smooth sumac occur on the sandy ridges above the springs level, and white poplar, red-osier dogwood, and peach-leaf willow on the flood plain around the stream outflow area.

The seven major plant associations of the Niobrara River valley provide a great 'outdoor classroom.'

Thus were left behind in the Niobrara River Valley, far from their nearest progenitors, three major remnant forest communities—eastern deciduous, Rocky Mountain pine, and northern boreal—intermingled or occurring within a stone's throw of each other. These botanical marvels would have been inundated had the proposed Norden Dam been constructed.

TALL-GRASS PRAIRIE

On the narrow floodplain of the Niobrara valley, at the foot of the forested slopes on both sides of the river, conditions became suitable for the development of a tall-grass prairie ecosystem. Several remnants occupying from a few to several hundred acres remain, but most are greatly disturbed, and some have been destroyed and reestablished. Post-glacial warming and drying conditions confined the tall grass to the flood plain patches, where they contain many "weedy" species as well as "typical" plants.

The major plant species of grasses and forbs occurring in this community are listed in Table 1.

UPLAND MIXED-GRASS PRAIRIE

On the Crookston table, above the north bluffs of the Niobrara valley, post-glacial conditions effected the establishment of a mixed-grass prairie ecosystem. This association contained many forage species, and was used by large herbivores, native Americans, and more recently, by cattle ranchers. Most of this tableland was used for haying, grazing, and in a few instances, crops. The seeding of bromegrass vastly changed the makeup of this community, but after being abandoned as ranchland, the mixed prairie association is returning. This community is bordered on the bluffs and ridges by the Rocky Mtn. pine forest, and on the opposite side by farms and ranchland.

The major plant species of grasses and forbs in this community are listed in Table 1.

PLEISTOCENE TERRACE/ MIXED-GRASS PRAIRIES

The rapid down-cutting of the Niobrara river in its limited meanders left behind flat pieces of tableland (old floodplains) at various elevations on the north side of the valley. The highest terraces adjacent to the Niobrara River were formed in early to mid Pleistocene, and were related to glacial events to the east. The dominant upper terraces were associated with the Wisconsin glaciation. The lower terraces represent local Holocene (post-Wisconsin) meanderings of the river (5). The largest of these terraces is the Huddle table, a fairly flat cropland/hay area of about 500 acres; numerous smaller terraces occur along the north valley escarpment. The main ones upon which nearly natural communities remain occur at 200 to 300 ft. above the level of the river, each with a different plant species mix, but with the features of a mixed-grass prairie. The common feature of these terraces are little bluestem and needle-and-thread grasses throughout, and downy brome in most disturbed areas. These Pleistocene terraces are bordered on the upper edges by the Rocky Mtn. pine forest, and on the lower side by grassy but weedy, gravelly slopes with scattered trees

extending down to the floodplain.

The major plant species of grasses and forbs in these terrace communities are listed in Table 1. Disturbed places on these terrace, such as roads, gullies, or bare areas, are populated by invaders: Rocky Mtn. beeplant, hoary vervain, curly-cup gumweed, mare's-tail, and eastern redcedar (2).

Sandhills Transitional Mixed Prairie

The transitional Sandhills prairie began to develop upon the Valentine sand when plants began invading the dunes, and increasingly less new dune formation occurred. Thus during the post-glacial period in north central Nebraska, a loose assemblage of mixed prairie species south of the Niobrara River evolved into the Sandhills prairie ecosystem. This is perhaps the most diverse plant association along the valley, with more total species and fewer dominant species than any other community there. The Sandhills prairie adjacent to the Niobrara River occupies the sculptured dunes and gravelly ridges above the eastern deciduous forest community on the south of the valley.

The major plant species of grasses and forbs in the Sandhills prairie can be seen in Table 1. The endangered "blowout bluebell", (Penstemon haydeni), has been observed in active blowouts, but its current status is questionable.

ADDITIONAL FEATURES

The table listing of species is restricted to those which are common, frequent or abundant, or if less frequent, are conspicuous. Common forbs and grasses were not listed in forest communities. Weedy species common to disturbed ground have also been omitted. In addition to the seven major plant associations described above, another distinct ecosystem in the Niobrara River Valley is the sandbar-marsh community along the south bank of the stream, extending from Fairfield Creek to Plum Creek. These sandbars provide nesting sites for the federally endangered least tern and piping plover; the marshes are very diverse in both aquatic flora and fauna. The four prairie communities of the Niobrara Valley and the Sandbar/Marsh ecosystem occur adjacent to, and often intermingled with, the three forest communities occupying this stretch of the Niobrara Valley.

Another unique feature of the Niobrara River at the Niobrara Valley Preserve is a very prolific fossil quarry on the bluff overlooking the Norden Bridge. This site has yielded 16 species found nowhere else, and in all a total of 88 Tertiary and recent species—an irreplaceable treasure of paleontology(7). Fossils from this site prove that Nebraska was once a tropical region inhabited by rhinoceros and camel.

EDUCATIONAL ACTIVITIES

Field studies have been conducted in the general region of Valentine for more than a decade, at the Valentine Lakes N. W. R., Merritt Reservoir and the Snake River, Ft. Niobrara N. W. R., and McElvie Natl. Forest, as well as eastward along the Niobrara River. The proposal to build a dam south of Norden gave impetus to studies identifying the flora and fauna of the region, and its acquisition by the Nature Conservancy made the area available for studies by Wayne State and other colleges in the area. The kinds of field classes conducted by Wayne State College faculty include General Ecology (on a seasonal basis); Plant Community studies—makeup and general relationships; Species diversity and indices of specific habitat types, both flora and fauna; Invertebrate Zoology study, mostly conducted in marshes from the Norden Bridge west to Fairfield Creek; Field Studies in Natural History, Geologic history and Ecology of the Niobrara Valley; and field study of specific organisms in Vertebrate Zoology, Ornithology, Mammalogy, and Advanced Plant Biology classes. Also, studies of interrelationships inherent in Ecology, such as bison cow/calf, rodent/habitat, water analysis, faunal movement and territoriality, have been conducted.

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Table 1.—Plant Species Occurence and Abundance In the Niobrara Valley Preserve and Vicinity (2, 3, 6).

Plant Species:		Plant Community 1 EDF	2 RMF	3 NBF	4 TGP	5 UMP	6 PLP	7 STP
GP. A—FERNS/FERN ALLIES:								
Equisetum spp.	**	3		4				
Botrychium spp.		2		3				
Cystopteris fragilis		4		4				
Thelypteris palustris			3					
Woodsia oregonia		2	2	3				
Selaginella rupestris						5	4	4
GP. B-GYMNOSPERMS:								
Juniperus horizontalis (creeping juniper)			2					
Juniperus virginiana (eastern redcedar)		4	4	4				
Pinus ponderosa (Ponderosa pine)		2	5	2				
GP. C—BROADLEAF TREES:								
Acer negundo (boxelder)		3		3				
Betula papyrifera (paper birch)				4				
Ostrya virginiana (ironwood)		3		4				
Quercus macrocarpa (bur oak)		4		2				
Juglans nigra		3		3				
Fraxinus pennsylvanica (green ash)		3		3				
Popilis alba (white poplar)		2		3				
Populus deltoides (cottonwood)		2	3	2	3			
Populus tremuloides				2				
Salix amygdaloides				3				
Tilia americana (basswood)		3		3				
Celtis occidentalis (hackberry)		3		3				
Ulmus americana (American elm)		3		2				
GP. D—BROADLEAF SHRUBS/VINES:								
Rhus aromatica (aromatic sumac)		3	4	2				3
Rhus glabra (smooth sumac)		2	4	3		3		3
Toxicodendron rydbergii (poison ivy)		3	2	3		2	2	4
Sambucus canadensis (common elderberry)		2	3	2				
Corylus americana (hazelnut)				3				
Symphoricarpos occidentalis (snowberry)		3	3	3	2	3	3	3
Ribes americanum		3	3	2				
Ribes missouriense (nor.gooseberry)		3	3	3				
Ribes odoratum		3		3				
Physocarpus opulifolius (prairie ninebark)		2		3				
Prunus americanus (wild plum)		3	3	3	2			
Rosa arkansana (wild rose)		2	3	2	2	3	3	3
Rubus occidentalis (black raspberry)		3	2	3				
Xanthozylum americanum (prickly-ash)		3	3	2				
Hypericum majus				3				
Cornus stolonifera		3		3				
Celastrus scandens (bittersweet vine)		2	3	2				
Parthenocissus vitacea (woodbine)		3	3	3				
Vitis riparia (riverbank grape)		3		4				
Shedherdia argentea (buffaloberry)		2	3					2
GP. E—GRASSES:								
Agropyron cristatum (crested wheatgrass)		2			3			
Agropyron smithii (western wheatgrass)					3	3	3	
Agrostis scabra		3	3	3				
Andropogon hallii (sand bluestem)						3	2	4
Andropogon gerardi (big bluestem)					3	2		2
Schizachyrium scoparius (little bluestem)					4	5	4	
Aristida purpurea (purple three-awn)		2				4	3	2
Bouteloua curtipendula (sideoats grama)					3	4	4	4
Bouteloua gracilis (blue grama)						3	3	3

Plant Species:	Plant Community 1 EDF	2 RMF	3 NBF	4 TGP	5 UMP	6 PLP	7 STP
Bouteloua hirsuta (hairy grama)					3	2	3
Bromus inermis (planted brome)					5		
Bromus tectorum (downy brome)				4	5	3	3
Calamovilfa longifolia (prairie sandreed)				5	5	5	
Catabrosia aquatica			3				
Dicanthelium oligosanthes (Scribner panic-gr)	3				3	3	2
Dicanthelium wilcoxianum		3			3		
Elymis canadensis (Canada wildrye)	3			3			
Elymus villosus	3			2			
Eragrostis spectabilis (purple lovegrass)				2	3	3	
Eragrostis trichodes					2		3
Festuca obtusa	3		3				
Festuca octoflora (nodding fescue)					3	3	3
Koeleria pyramidata (prairie junegrass)				3	3	3	
Muhlenbergia cuspidata (plains muhly)				3	2	3	
Muhlenbergia pungens (sandhill muhly)						3	
Muhlenbergia racemosa	3	3	3				
Oryzopsis micrantha	2	2	3				
Panicum capillare					2	3	3
Panicum virgatum (switchgrass)				3	2	2	3
Paspulum setaceum				2	3	3	4
Redfeldia flexuosa (blowoutgrass)							4
Sorghastrum nutans (Indiangrass)				4			
Sporobolus cryptandrus (sand dropseed)					3	2	3
Stipa comata (needle-and-thread grass)					4	4	5
Stipa spartea					3	2	3
Stipa viridula (green needlegrass)					3	2	
GP. F—OTHER MONOCOTS:							
Commelina erecta	2			2			2
Tradescantia occidentalis					3	3	3
Carex spp. (sedges)	3	3	3	3	3	3	3
Cyperus spp. (sedges)				3	3	3	3
Eliocharis spp.							3
Habenaria hyperborea	3		3				
Spiranthes cernua	3			2			
Yucca glauca (small soapweed)					3	2	3
GP. G—DICOT FORBS:							
Berula arecia			3				
Sanicula canadensis	3						
Aralia nudicaulis			3				
Achillea millefolium (common yarrow)				2	3	3	2
Asclepias arenaria					2	2	3
Asclepias incarnata			3				
Asclepias stenophylla					2	2	2
Asclepias verticillata				2	2	3	2
Antennaria neglecta (pussytoes)	3	2					3
Antennaria parvifolia					3	3	3
Artemesia campestris (prairie sage)					3	3	3
Artemesia frigida (fringed sage)				2	4	3	4
Aster ericoides (heath aster)	4	3		4	3	3	3
Aster laevis	3	3	2				
Aster oblongifolius (aromatic aster)		3			3	3	
Aster nova-angliae (New England aster)			3				
Aster simplex				3			
Chrysopsis stenophylla					3	2	
Corsium canescans					3	3	3
Echinacea angustifolia					3	3	3
Eupatorium perfoliatum				2			
Euthamia gymnospermoides				3			
Gaillardia puberula (Indain blanketflower?)			3	2	2		
Grindelia squarrosa					3	4	2

Plant Species:	Plant Community 1 EDF	2 RMF	3 NBF	4 TGP	5 UMP	6 PLP	7 STP
Gutierrezia sarothae (broom snakeweed)					2	3	3
Haplopappus spinulosus (spiny goldenweed)					3	3	3
Helianthus annuus (annual sunflower)				4			
Helianthus nuttallii (Nuttall's sunflower)			3				
Helianthus rigidus (stiff sunflower)					3	3	3
Helianthus petiolaris						2	3
Heliopsis helianthoides (rough ox-eye)	2			3			2
Hymenopappus tenuifolius (old-plainsman)					3	3	3
Lactuca spp. (lettuce?)	2			3			
Liatris aspera	3	2					
Liatris punctata (dotted gayfeather)					3	4	3
Liatris squarrosa (scaly blazing-star)				2	2	2	3
Lygodesmia juncea (skeletonweed)					3	3	3
Microseris cuspidata (false dandelion)				3	3		
Ratibida columnifera (upright prairieconeflower)2	2			4	3	4	
Rudbeckia hirta (black-eyed Susan)	2			3	2	3	
Senecio integerrimus	3	3	3				
Senecio plattensis (golden ragwort)	3			3		2	
Senecio riddellii (Riddell's groundsel)							4
Senecio tridenticulatus (squaweed)							3
Solidago canadensis (Canada goldenrod)			3				
Solidago missouriensis (Missouri goldenrod)					3	3	3
Solidago nemoralis (gray goldenrod)					3	3	3
Solidago rigida (Solidago rigidus)			2	3	2		
Thelesperma filifolium					4	4	2
Impatiens capensis			4				
Lithospermum carolinense (hairy puccoon)	3				3	3	3
Lithospermum incisum				2	2	3	2
Draba reptans					3	3	
Erysimum asperum (western wallflower)				3	3	3	
Erysimum cheiranthoides	3	2					
Lesquerella ludoviviana (silvery bladderpod)					3	3	3
Opuntia spp. (compressa = prickly-pear)				2	3	3	3
Campanula americana	2		3	2			
Campanula rotundifolia	2		3				
Triodanis perfoliatum				2	3	3	3
Cleome serrulata (Rky. Mt. beeplant)			3				
Criststella jamesii					3	2	3
Arenaria laetiflora	3	3	2				
Cycloloma atriplicifolium (winged pigweed)			2	3	3	3	
Ipomoea leptophylla (bush morningglory)				3	2	3	
Amorpha canescens (leadplant)				3	2	3	
Amphicarpa bracteata	3			3			
Astragulus crassicarpus (prairie ground-plum)				3	3		
Dalea candida (white prairie-clover)					3	3	3
Dalea ennandra (slender dalea)					3	4	3
Dalea purpurea (purple prairie-clover)				2	3	3	3
Dalea villosa (silky prairie-clover)					2	2	4
Desmodium canadense	3			2			
Desmodium glutinosum	3		3				
Calylophus serrulatus (yellow evening-primrose)			2	3	3	3	
Glycyrrhiza lepidota				3			
Lathyrus polymorphos (showy vetchling)					2	3	3
Oxytropis lambertii (Lambert crazyweed)				3	3	4	
Psoralea argophylla (silverleaf scurfpea)			3		3	3	3
Psoralea digitata (digitate scurfpea)					3	2	3
Psoralea esculenta (prairie turnip)				2	3		
Psoralea lanceolata (lemon scurfpea)				3	2	3	
Hedeoma hispida (rough pennyroyal)					4	3	2
Monarda fistulosa	3	2	3				
Monarda pectinata					2	2	3

Plant Species:	Plant Community 1 EDF	2 RMF	3 NBF	4 TGP	5 UMP	6 PLP	7 STP
Linum rigidum (stiffstem flax)					3	3	3
Mentzelia nuda (desert-lily)					2	2	3
Circaea lutetiana	3		3				
Gaura coccinea (scarlet gaura)					2	3	
Gaura parviflora					4	3	
Oenothera biennis (evening-primrose)			3				
Oenothera nuttallii					2	2	3
Oenothera rhombipetala (diamond evng-primrose)					3	3	3
Orobanche fasciculata					3	3	3
Oxalis dillenii	3		3				
Plantago patagonia					3	3	3
Collomia linearis							3
Ipomopsis longiflora (white gilia)						3	
Eriogonum annuum (umbrella-plant)				3	2	4	
Rumex venosus (veined dock)							3
Talinum parviflorum							3
Androsace occidentalis	3	3			3	2	
Anemone cylindrica	2			3			
Anemone patens		3					
Aquilegia canadensis	3		3				
Delphinium virescens (prairie larkspur)				2	3	3	3
Thalictrum dasycarpum	3						
Fragaria vesca	3		3				
Potentilla arguta (tall cinquefoil)				3	3	3	3
Galium aparine	3	3	3				
Commandra umbellata (bastard toadflax)					3	3	3
Heuchera richardsonii	3	3	4				
Mimulus glabrata			3				
Penstemon (albidus = sm. wh. beard-tounge)					3	3	3
Penstemon angustifolius (narrowleaf beard-tounge)					3	3	4
Penstemon gracilis		2			3	3	
Penstemon grandiflorus (shell-leaf penstemon)		2	3	3	4		
Scrophularia lanceolata	3		2				
Laportea canadensis			4				
Praietaria pennsylvania	3						
Pilea pumila			3				
Urtica dioica	3		4				
Phryma leptostachya	3		3				
Verbena stricta (hoary vervain)	3	2		3	3	3	3
Verbena urticifolia	3		3				
Viola nephrophylla	3		3				
Viola pratincola	3		3				

*Plant Communities: 1, Eastern Deciduous Forest; 2, Rky. Mtn. Pine Forest; 3, Northern Boreal (Birch) Forest; 4, Tall-grass Prairie; 5, Upland Mixed Prairie; 6, Pleistocene Terrace Mixed Prairie; and 7, Sandhills Transitional Prairie.

**Index of abundance: 5, abundant; 4, numerous or frequent; 3, common; 2, ocassional or infrequent; 1, rare. Only those species which were common in one or more communities are listed in the table. Exceptions are in-frequent but conspicuous tree species.

BIOLOGY OF AN ENZYME: A RESEARCH-LIKE EXPRIENCE FOR INTRODUCTORY BIOLOGY STUDENTS

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The primary motivation for constructing this series of laboratory exercises was a strong desire to introduce a realistic experience in biological research that was nevertheless feasible with substantial numbers of beginning biology majors. Previous experience with completely individualized projects at the introductory level suggested that a highly structured approach with built-in decision points might provide a workable model. We had already initiated a major faculty-student research project centering on alkaline phosphatase in crustacean gill (Lovett et al., 1989) and were struck by the adaptability of our experimental design to the teaching laboratory situation.

We designed the laboratory exercises to represent real research in the sense that the expected experimental outcomes are not known by either instructor or student. True hypothesis testing can thus take place with plentiful opportunity for "failure", an important ingredient in doing science. By starting with a research area in which departmental faculty and upper-level students are already engaged, the beginning students quickly perceive that they are involved in a current research question. Experiments are not simply repetitions of classic demonstrations. A study of the biology of alkaline phosphatase offers a multitude of questions centering on the characterization of a specific tissue function and the effects of environmental factors on that function. The choices of experimental organism and tissue source are made by students in consultation with the instructor, based on an original premise that tissue involved in solute transport may contain high levels of the enzyme.

A second major factor in designing a research-like experience was a desire to represent the process of research as accurately as possible. Thus we decided to extend the laboratory exercises over several weeks and to a limited extent established experimental designs on the basis of previous results. Repetitive measurements billed up front as being "tedious" reveal to students the true nature of much of scientific research. Some students balk at this "boring" approach but others welcome the opportunity to become expert at the useful technique of measuring enzyme activity. The concept of reproducibility is experienced first-hand as student teams attempt to replicate earlier results. And taking responsibility for one's experimental decisions is another valuable lesson. Further responsibility is developed by including a session on solution preparation, in which each team prepares a buffer for use later by the entire class.

We felt that the logistics of operating a research-based laboratory for an introductory course had to be straightforward, using a minimum of equipment and supplies. The enzyme labs require waterbaths, student spectrophotometers, micropipettors, and a small set of reagents. A balance, pH meter, and preparative centrifuge are required for individual exercises. Microcomputers are employed throughout to facilitate computations and graphing but these are optional.

The major outcome of this approach centers on the improved preparation of our students for upper-level courses and for independent research. The number of Lake Forest College biology students participating in summer research has grown from one in 1988 to 15 in 1991, partly as a result of an early experience demystifying the process of research. Students can easily visualize themselves in

research and thus it more readily becomes a part of their intellectual life. By the fourth week of this five-week series, the students grow quite confident of their manipulative skills in the laboratory. Instructors thus spend less time teaching technique and more time consulting with students on experimental design and data analysis. Students' evaluations have been consistently positive: "I'm very enthused about going to lab", "students are encouraged to think and problem-solve", "I find it interesting that we are doing relatively novel things in lab and maybe finding things that had never been found before", "the lab forces one to start asking why and think about what one is doing", "the experiments are really cool".

Following are the laboratory instructions provided to each student prior to the laboratory session. Instructors are very explicit in what we are trying to accomplish in terms of setting up a research-like experience. The design can be adapted to a wide variety of research problems. It may be important to develop such a design based on specific departmental faculty expertise. A brief account of this laboratory design has appeared recently (Towle, 1991).

ALKALINE PHOSPHATASE I: BUFFERS AND STANDARDS

INTRODUCTION

Chemical reactions in living organisms occur rapidly at moderate temperatures and under mild conditions primarily because of the catalytic action of specialized proteins called enzymes. Each step in a chain of biochemical reactions is usually catalyzed by a specific enzyme. The lack of even one enzyme can prove fatal. Galactosemia, for example, is a human genetic disease caused by a deficiency of the enzyme phosphogalactose uridyl transferase. This disease can result in early death if it goes undetected.

The actual catalytic site of an enzyme molecule is a small area where the enzyme's component amino acids are arranged to precisely "fit" its substrate, which is the substance acted upon by the enzyme and changed to the product. The rate of an enzyme-catalyzed reaction depends partly on how well the enzyme and substrate fit together. Thus an environmental factor (such as pH or high temperature) which might change the shape of either enzyme or substrate could alter the rate of reaction. In addition the concentration of active enzyme and substrate molecules would be expected to have important effects on the rate.

The rate of an enzyme-catalyzed reaction may be measured by (1) the disappearance of substrate, or (2) the appearance of product. The enzyme and substrate are mixed and allowed to react for a certain time period; the amount of either the substrate or the product is then measured. This amount gives the rate of activity of the enzyme per unit of time.

Alkaline phosphatase, the enzyme we will be studying, is important in recycling phosphate within living cells and tissues. It seems to be particularly prevalent in tissues which are transporting nutrients, including intestine and kidney. This enzyme catalyzes the cleavage of a phosphate group from a variety of compounds, including the "artificial" substrate used here, p-nitrophenyl phosphate. This substrate is colorless. However, one of the products, p-nitrophenol, is yellow in basic solutions. The appearance and intensity of yellow color thus indicates the degree to which the substrate has been acted upon by the enzyme.

In the clinical setting, elevated blood levels of alkaline phosphatase coincide with certain types of cancer, particularly cancer of bone, liver, and lung. Thus measurements of alkaline phosphatase activity are important in medical diagnosis and are done

routinely as part of a typical physical examination. Research on alkaline phosphatase at Lake Forest College has centered on its role in transporting tissues, primarily the gill of crustaceans. Environmental salinity seems to be a cue in regulating levels of alkaline phosphatase. Precisely what its function may be, however, is unknown.

METHODS AND MATERIALS

In this first laboratory, we will be laying the experimental groundwork for subsequent laboratories. We will (1) determine the absorption spectrum of the yellow p-nitrophenol, (2) measure the relationship between the amount of p-nitrophenol and its absorbance of light, and (3) prepare buffers for use in subsequent laboratories. Because of a limitation on the number of balances and pH meters, the last task should be rotated among groups rather than done all at one time.

General Laboratory Methods

Cleanliness is next to godliness! To work efficiently and accurately in a biology laboratory, you must learn careful technique. You should keep in mind that tabletops, clothing, and particularly your hands are chemically quite dirty, even though you may have just cleaned them. Anything that will come in contact with experimental chemicals and solutions must avoid contact with tabletops and hands. Use clean Kimwipe tissues to handle stirring bars and similar items. Do not touch the "working end" of any pipet or pipettor tip. If you must lay a pipet on the table for reuse, lay it on a Kimwipe. All glassware must be rinsed in tap water (5-6 times) and distilled water (4-5 times), and dried in a manner to keep it clean.

Pipetting is the route to most quantitative data! Measuring small amounts of liquids must be done very carefully to obtain repeatable quantitative data. Remember to keep the working ends clean. All styles of pipets and pipettors must be kept upright when containing liquid, to prevent the liquid from running back into the pipetting device and contaminating subsequent measurements. (The pipettor can also be seriously damaged!) Pipetting devices work best when you are patient with them. Use slow rather than abrupt movements both when taking a sample into the pipet and dispensing it. Watch for and avoid bubbles. It is better to be very careful the first time than to repeat an entire series of measurements because of carelessness. After pipetting a sample into a larger volume of solution, be sure to mix well by vortexing or stirring. The two most common sources of error in quantitative work is careless pipetting and incomplete mixing.

Personal safety. We will do absolutely no pipetting by mouth. If materials are caustic or toxic, we will recommend eye protection, gloves, and/or lab coats. Be sure you know where the fire extinguisher and safety equipment are located. Of course there will be no eating or drinking in the laboratory. In the operation of equipment, be sure you are familiar with its dangers before operating it. Ask if you are unsure!

Use of the Spectronic 21D Spectrophotometer

The amount of p-nitrophenol resulting from the enzyme-catalyzed hydrolysis of p-nitrophenyl phosphate is measured using a spectrophotometer, an instrument capable of detecting the amount of light absorbed by colored solutions. According to Beer's law,

$$A = E \cdot l \cdot c$$

where A is the measured absorbance, E is an empirical constant (the extinction coefficient), l is the length in centimeters of the light path through the sample solution, and c is the concentration of absorbing material in moles/liter. The amount of light absorbed (absorbance) is thus directly proportional to the concentration of absorbing material.

Follow these steps to operate the spectrophotometer:

a. **APPLY POWER**—Turn on the power switch. Allow the instrument at least five minutes to warm up.

b. **SELECT THE WAVELENGTH**—Turn the wavelength control to the desired wavelength in nanometers (10^{-9} meters).

c. **SET SENSITIVITY** to "M" and **MODE** to "Absorbance".

d. **ADJUST ZERO ABSORBANCE**—Add at least 3 milliliters (ml) of "blank" solution (may be water or buffer, depending on the experiment) to a clean cuvet (a special test tube). Wipe fingerprints from the outside of the cuvet and insert it all the way into the cuvet holder, aligning the mark on the cuvet with the mark on the holder. Adjust the knurled knob marked "Increase" until the digital display reads zero absorbance. Remove the blank cuvet and place in a non-metallic test tube rack for later use.

e. **MAKE A SAMPLE MEASUREMENT**—Add at least 3 ml of the sample solution to be measured to a clean cuvet, wipe the outside of the cuvet, and insert the cuvet into the holder, aligning marks as before. Now read and record the absorbance. Treat the cuvets with care to avoid scratching them.

Determination of the Absorbance Maximum

The first task will be to construct an "absorbance spectrum" to determine the wavelength at which the colored substance (in this case p-nitrophenol) demonstrates maximum absorbance. This wavelength will then be used in all subsequent measurements to optimize sensitivity of the spectrophotometer.

To the "blank" cuvet, add 3 ml of a solution of 0.04 M sodium hydroxide (NaOH). Starting with a wavelength of 360 nm, use the blank cuvet to set zero absorbance as described above.

To the "sample" cuvet, add 1 ml of a solution of 0.01 mM p-nitrophenol plus 2 ml 0.04 M NaOH. Then take an absorbance measurement of the sample at 360 nm wavelength. Record the wavelength and absorbance value in your laboratory notebook, using a tabular format. Remove the sample cuvet.

Adjust the wavelength to 370 nm and repeat steps c, d, and e above. Increase the wavelength in steps of 10 nm and repeat your measurements until you are satisfied that you have found the absorbance maximum.

Construction of a Standard Curve

Using 15-ml test tubes, set up a standard dilution series in duplicate (two tubes of each) as follows, pipetting as carefully as possible:

0.04 M NaOH	0.01 mM p-Nitrophenol in NaOH
4.0 ml	1.0 ml
3.0	2.0
2.0	3.0
1.0	4.0
0.0	5.0

Mix thoroughly by careful vortexing. (Ask for a demonstration!)Using the wavelength which produces maximum absorbance, adjust zero absorbance using your "blank" cuvet. Then measure the absorbance of each of the standard dilutions by pouring the contents of each test tube into a cuvet and reading the absorbance, rinsing with distilled water between each reading. Be sure to wipe fingerprints between each reading and align the marks properly. Record your data in tabular format in your laboratory notebook.

Making Buffers

Making solutions accurately is one of the most important skills in any biology laboratory. Remember cleanliness and careful measurement! Also remember that a 1 molar (M or moles/liter) solution contains 1 gram molecular weight of solute made up to one liter with distilled water. Nearly all other solutions can be deduced from that one rule. (One exception: a 1% (w/v) solution contains 1 gram of solute per 100 ml of solution.)

You and your partner should make up one of the buffer solutions to be used by your class in future experiments. We will check your product for proper pH. Select one of the pH values desired (pH 8.5, 9.0, 9.5,10.0, 10.5, and 11.0).

The desired buffer is 0.1 M 2-amino-2-methyl-1-propanol (MW 89.1, a liquid) dissolved in 0.001 M $MgSO_4$-$7H_2O$. Although you have been given the molecular weight of the first solute, you should calculate the molecular weight of the magnesium sulfate crystals (be sure to include the waters of crystallization).

Ask for a demonstration of the balance with which you will weigh out the proper amounts of 2-amino-2-methyl-1-propanol and magnesium sulfate to make one liter of buffer. Calculate the weight in grams for each solute and check with the instructor before proceeding. Describe your procedure in your laboratory notebook. Using a clean spatula (for powder) or clean pipet (for liquid), weigh out the two substances into a beaker and add distilled water (considerably less than 1 liter). Stir to dissolve.

Ask for a demonstration of the pH meter with which you will adjust the pH of the buffer. Be very careful of the pH electrode; it is quite fragile. The pH meter should be standardized with standard buffer once a day. With the stirrer spinning and the lower centimeter of the electrode immersed in the solution, take continuous pH readings. With a pasteur pipet, add 10% NaOH to raise the pH, concentrated HCl (use in hood: careful!!) to lower the pH. Wait for the pH to stabilize after each addition. Near the desired pH value, add very small volumes of NaOH or HCl until the desired value is obtained.

Remove the pH electrode, rinse it off with distilled water and replace it in the electrode storage solution.

Pour the pH-adjusted buffer carefully into a 1-liter volumetric flask, using a funnel if necessary. Rinse out the beaker with a small amount of distilled water and add that to the flask as well. Now bring the volume up to the mark on the neck of flask, using distilled water. The bottom of the meniscus should match the mark exactly.

Label a storage bottle (plastic or glass) with tape containing the concentrations of the solutes, your name, section number, and date. Refrigerate until needed in a subsequent lab period.

RESULTS

Plot the absorbance spectrum on a linear graph with the wavelength as the x-axis and the absorbance as the y-axis. Use graphics software to plot your graph via computer and laser printer, as demonstrated by the instructor. Print out three copies of your graph on the laser printer, one for each member of your lab team and one for the instructor. Retain your graph for inclusion in your laboratory report, and submit a copy of your original data plus your graph to the lab instructor before you leave for the evening. If you wish to save your graphs for future use, bring a formatted floppy disk to the laboratory session.

For your standard curve, calculate the micromoles of p-nitrophenol present in each of the 5-ml volumes. (How many moles is contained in 1 ml of a 0.01 mM solution?) Plot these value on the x-axis of a linear graph and the corresponding absorbance values on the y-axis. Again use graphics software. If the standard "curve" is linear, calculate the slope of the curve as absorbance units per micromole. What is the percent variation between your duplicate values? How accurate is your pipetting? Analytical skills need to be developed to the point where less than 5% difference exists between duplicate determinations. Make three copies of your standard curve graph, submitting one with your copy of original data and calculations.

In your report, describe how you would make a buffer consisting of 10 mM Tris and 1 mM EDTA, pH 8.0.

ALKALINE PHOSPHATASE II: FRACTIONATION AND ASSAY

INTRODUCTION

In this second week of our work on alkaline phosphatase, we will learn about homogenizing and fractionating tissue and will also carry out a measurement (assay) of alkaline phosphatase on each fraction. Homogenizing breaks up tissues and cells into organelles, such as nuclei, mitochondria, membranes, etc. After homogenization, we are able to handle the tissue with a pipet, measuring out precise quantities and using the material as a source of enzyme activity. Fractionation will be accomplished using a refrigerated centrifuge capable of spinning a rotor at 20,000 rev/min. The different cell organelles have different densities which permit separation by centrifugation at various forces of gravity.

The measurement of alkaline phosphatase is done by incubating an enzyme source with the substrate, p-nitrophenyl phosphate, in one of the buffers which you made during the previous lab period. Temperature will be controlled by a water bath set at 37C, the optimum temperature for most enzymes. The reaction is initiated by adding a small volume of homogenate or tissue fraction and is stopped by adding sodium hydroxide which will denature the enzyme and also provide a basic solution for maximum yellow color of one of the products, p-nitrophenol.

As described in the previous handout, different tissues are expected to have different levels of alkaline phosphatase activity. Although the specific biological function of alkaline phosphatase is not well understood, various investigators have reported that tissues involved in nutrient transport often contain high levels of alkaline phosphatase. With each team selecting a different tissue, we should be able to produce data which might confirm or argue against this observation. Give careful thought to your choice of experimental material. You will be working with it for the next several laboratories.

Remember: Careful pipetting and mixing are the clues to great results!

METHODS AND MATERIALS

FRACTIONATION

1. *Obtaining the tissue:* Cool a homogenizer by placing it in crushed ice. Anesthetize and sacrifice your experimental organism with humane methods. Consult the instructor for details of appropriate humane treatment. In our case, a frog will be anesthetized on ice and then pithed by destroying the central nervous system.

Place approximately 1.5-gram samples of the desired tissue in about 10 ml of ice-cold homogenizing medium (250 mM sucrose, 6 mM EDTA, 10 mM Tris, pH 7.8 with HEPES) in a labelled beaker on ice. Each team should work with a different tissue. Place the animal remains in a plastic bag in the freezer.

2. *Homogenizing the tissue:* With forceps, remove the tissue from the beaker, blot on Kimwipes, and quickly weigh to the nearest 0.1 gram. Place the tissue into the ice-cooled homogenizer. Multiply the weight of the tissue in grams by 10 and add that many milliliters of cold homogenizing medium to the homogenizer. Homogenize by hand until no tissue fragments are apparent, keeping the homogenizer in ice as much as possible. Place two single thicknesses of cheesecloth over a small beaker on ice, and carefully pipet the homogenate onto the cheesecloth so that large tissue fragments are trapped on the cloth and the remaining homogenate is filtered into the beaker. "Wring out" the cheesecloth with the large end of a Pasteur pipet. Pipet exactly 10 ml of the resulting homogenate into a numbered plastic centrifuge tube (for the DuPont-Sorvall RC5C centrifuge) and keep on ice. Record the number. Place the remaining homogenate in a labelled plastic test tube and keep on ice.

3. *Preparation of the nuclear fraction:* The instructor will collect the centrifuge tubes from all teams and centrifuge them at 1,000 gravities for 10 minutes (at 2-4 C). After the centrifugation is completed, very carefully remove the upper supernatant with a 10-ml pipet and place it in a clean numbered centrifuge tube. Record the number. You should leave some supernatant behind rather than disturb the pellet of nuclei and unbroken cells. Give the new centrifuge tube to the instructor for the second centrifugation.

Using a stirring rod, resuspend the nuclear pellet in 10 ml of fresh homogenizing medium. If fragments are visible, use your rinsed-out homogenizer to disperse the fragments. Place the resuspended nuclear fraction in a labelled plastic test tube on ice.

4. *Preparation of the mitochondrial fraction:* The instructor will again collect the centrifuge tubes from all teams and centrifuge them at 10,000 gravities for 30 minutes (at 2-4 C). After the centrifugation is completed, remove the supernatant with a 10-ml pipet and place it into a labelled plastic test tube. This supernatant will be the "microsomal and soluble fraction".

Resuspend the mitochondrial pellet in 10 ml of homogenizing medium, using a stirring rod and homogenizer if necessary. Keep both the microsomal and soluble fraction and the mitochondrial fraction on ice.

ASSAY OF ALKALINE PHOSPHATASE

1. *Setting up assay tubes:* While the second centrifugation is progressing, you should set up thirteen large glass test tubes as follows, pipetting buffer (use pH 10 for this assay) and substrate (0.01 M p-nitrophenyl phosphate) into labelled test tubes. You may also pipet the p-nitrophenol (the standard) into tubes 11 and 12. Quantities are indicated in microliters. Remember that 1 ml = 1000 microliters (µl). Use the P-1000 micropipettor and blue tips for 500 µl, and the P-200 micropipettor and yellow tips for 100 and 200 µl. In adjusting volume, **do not** turn the knurled adjuster past the maximum volume of the pipettor (1000 for P-1000 and 200 for P-200). The set volume is obtained by depressing the main plunger to the first stop and slowly raising the plunger after placing the end of the disposable tip into the solution. Transfer the solution to the desired destination by slowly depressing the plunger to the second stop. **Do not** hold the pipettor with the tip above horizontal, to avoid contaminating the pipettor. Work the plunger slowly to get good reproducibility.

After adding buffer and substrate, place the test tubes in a plastic rack in a water bath at 37 C.

2. *Starting the assay:* When all of your tissue fractions are prepared and waiting in an ice bucket, start the alkaline phosphatase reaction by carefully adding the indicated volume of each fraction, waiting 30 seconds between each addition. Use a fresh pipet tip for each different fraction. Vortex the test tube to mix enzyme source with substrate and buffer, and place the test tube back in the water bath at 37°C. Continue in this way until all 13 tubes are incubating.

Tube Number	B1	1	2	3	4	5	6	7	8	9	10	11	12
Buffer pH10	500	500	500	500	500	500	500	500	500	500	500	500	500
Substrate	500	500	500	500	500	500	500	500	500	500	500	500	500
Homogenizing medium	100												
Homogenate		100	100	200	200								
Nuclear fraction						100	100						
Mitochondrial fraction								100	100				
Microsomal and soluble fraction										100	100		
0.5mM p-Nitrophenol (Note concentration)												100	100

3. *Stopping the reaction:* Exactly 20 minutes after the first addition of homogenizing medium to the buffer-substrate mixer, stop the reaction by adding 4.0 ml of 0.04M NaOH from a dispenser. Vortex the test tube and place it back in the rack. At 30-second intervals, continue to add NaOH to one tube at a time until all 13 tubes have been treated. Thus each incubation will last for exactly 20 minutes!

4. *Reading the absorbance:* Refer to the previous lab handout describing standardization of the spectrophotometer, setting the wavelength at the point of maximum absorbance which you determined. Adjust zero absorbance using the contents of the "Bl" tube in a clean cuvet. Making sure that the contents of each tube are well mixed, pour at least 3 ml of the solution from tube 1 into a spectrophotometer cuvet. Wipe the outside of the cuvet and read the absorbance. Determine the absorbance of tubes 2-12 in the same way. Record your data in your notebook.

Be sure to label your homogenate and fractions carefully, place a rubber band around the tubes, and store in the freezer until the next laboratory.

RESULTS

Calculate the absorbance per micromole of standard p-nitrophenol, using the averaged data from tubes 11 and 12. Note that a 0.5 mM p-nitrophenol solution contains 0.5 micromoles/ml. This calculation assumes that the relationship between absorbance and p-nitrophenol concentration is linear, as investigated in the previous lab exercise. We are taking two points from that line (zero and the average of tubes 11 and 12) to calculate the slope of the line.

Now divide each of the remaining experimental absorbance values by the calculated absorbance/micromole. This will give you micromoles of p-nitrophenol produced per 20 minutes of incubation. Now divide these values by 20 to get micromoles of p-nitrophenol produced per minute. You are encouraged to use a spreadsheet for these calculations, although they can of course be done with a calculator.

Are the 0.2-ml values for homogenate twice those of the 0.1-ml incubations? Why or why not?

Express the alkaline phosphatase activity of each fraction as a percent of the total activity in the homogenate. Because we used the same volume (10 ml) throughout the preparation of the different fractions, this calculation is straightforward. Construct a bar graph to depict these data.

DISCUSSION

Comparing your values for the different fractions, which fraction contains the most alkaline phosphatase activity? How does this discovery match with your expectations? Compare your values with those of other teams working with different tissues. What can you tell about the likely function of the tissue you chose?

ALKALINE PHOSPHATASE III:
pH AND SUBSTRATE EFFECTS

INTRODUCTION

In the third week of our work on alkaline phosphatase, we will measure the effects of pH and substrate concentration on the functioning of the enzyme which you have prepared. The measurements require great attention to detail and are indeed tedious in and of themselves. However, the results that we can obtain will tell us much about the properties of the enzyme we are studying and in fact can tell us about the functioning of the tissue from which the enzyme was prepared.

The effect of pH on the activity of the enzyme can provide us with information about the nature of the essential amino acid side chains in the enzyme. Enzymes are held together by a combination of hydrogen bonds, hydrophobic interactions, disulfide bridges, and ionic attractions. It is this last type of bond which is affected most dramatically by pH. If the pH is lowered, the excess hydrogen ions can bind to a positively-charged side chain, neutralizing that charge and perhaps removing one of the partners in an ionic bond. The structure of the enzyme's active site may change as a result, and its catalytic efficiency can thereby be affected. As you might guess from the name, alkaline phosphatases from a wide variety of tissues tend to show an optimum pH in the basic range (above pH 7). The optimum pH can be used as a diagnostic device, describing the properties of the enzyme from a particular source. In addition, using the optimum pH for the second part of this laboratory will help to ensure that the effect of substrate will be measured under "ideal" conditions for the enzyme.

By varying the substrate concentration and measuring the rate of the enzyme catalyzed reaction, we will be able to construct a "Michaelis-Menten plot", graphing rate (in micromoles p-nitrophenol/minute) on the Y-axis against substrate concentration (in moles p-nitrophenylphosphate/liter) on the X-axis. The maximum rate will be determined as V_{max}, and one-half that value will be calculated. The Michaelis constant, K_m, is defined as the substrate concentration at one-half V_{max}. This K_m value is a very useful diagnostic estimate of the affinity between enzyme and substrate; the lower the K_m the higher the affinity and the more efficient the enzyme can be.

The measurement of alkaline phosphatase will be carried out in the manner employed in the previous experiment. Remember: careful pipetting and mixing are the secrets to great results!

Tube	B1	1	2	3	4	5	6	7	8	9	10	11	12
Buffers													
pH 8.5		500	500										
pH 9.0				500	500								
pH 9.5						500	500						
pH 10.0	500							500	500				
pH 10.5										500	500		
pH 11.0												500	500
Substrate	500	500	500	500	500	500	500	500	500	500	500	500	500
Dist H2O	100	0	0	0	0	0	0	0	0	0	0	0	0
Enzyme	0	100	100	100	100	100	100	100	100	100	100	100	100

Tube	B1	1	2	3	4	5	6	7	8	9	10	11	12
Buffer	500	500	500	500	500	500	500	500	500	500	500	500	500
Substrate	0	25	25	50	50	100	100	200	200	400	400	0	0
Dist H2O	500	475	475	450	450	400	400	300	300	200	200	400	400
0.5 mM p-nitrophenol	0	0	0	0	0	0	0	0	0	0	0	100	100
Enzyme	100	100	100	100	100	100	100	100	100	100	100	100	100

METHODS AND MATERIALS

Select one of your best tissue fractions from the previous experiment, one that gave substantial enzymatic activity. If your team selected a tissue that turned out to have very low activity, check with the instructor before proceeding. He/she may suggest that you borrow a tissue fraction from another team. Thaw out the tube containing your frozen tissue fraction in a beaker of tap water, and place in the tube in crushed ice for the duration of the experiment. Be sure that the material is very well mixed. If fragments are visible, use a homogenizer to disperse them.

Effect of pH on Enzyme Activity

Set up 13 test tubes according to the following table, using the buffers you made during the first laboratory period. (You finally get to use them!). Do not add enzyme (as usual) until you are ready to start timing the reactions. Remember that the volumes are in microliters. Pipet slowly, holding the tip against the inside of the test tube as you empty the tip.

Add buffers, substrate (0.01M p-nitrophenylphosphate), and water as indicated and place the test tubes in the 37C water bath. If your enzyme source is extremely potent, you may wish to dilute it with homogenizing medium so that your final results will be easily readable. Consult with your instructor if this is the case. When your tubes are ready, add enzyme at 30-second intervals, vortexing each tube and placing it back in the water bath. Exactly 20 minutes after the first addition of enzyme, add 4.0 ml 0.04 M NaOH to stop the reaction and vortex to mix. Continue adding NaOH to each tube in succession at 30-second intervals so that each incubation lasts 20 minutes.

Set your spectrophotometer at the optimum wavelength for p-nitrophenol. Add at least 3 ml of tube B1 to a cuvet, insert it into the cuvet holder, and adjust the display to zero absorbance. Pour at least 3 ml of incubation tube 1 into a clean cuvet, wipe the outside of the cuvet, insert it into the cuvet holder matching the marks, and read the absorbance to three decimal places. Without touching the adjustments, proceed to read the absorbance of each incubation tube in succession, pouring the waste into the large beaker provided.

Effect of Substrate Concentration

Set up a second set of thirteen glass test tubes as follows. Volumes are given in microliters. Add only the first four ingredients, then place the test tubes into a 37°C water bath. The buffer should be 0.1 M 2-amino-2-methyl-1-propanol in 0.001 M $MgSO_4$-$7H_2O$, using the pH value just determined as producing maximum activity. The substrate is 0.01 M p-nitrophenylphosphate.

Incubate the tubes in a 37C water bath. As before, add enzyme to the tubes at 30-second intervals, vortexing to mix. Exactly 20 minutes after the addition of enzyme, add 4.0 ml of 0.04 M NaOH and vortex to stop the reaction. Read the absorbance of each sample, using the contents of the B1 tube to set zero

absorbance. Use the standards (tubes 11 and 12) from the substrate run to make your calculations. Place your enzyme source back in the freezer for later use.

RESULTS

Ask for a demonstration of spreadsheet software. You may use either spreadsheet or graphics software to produce the graphs you will need, but you should learn enough about spreadsheet software to do the calculations.

Calculate the alkaline phosphatase activity for each of your incubation tubes, knowing the micromoles of p-nitrophenol present in the standards and calculating the micromoles of p-nitrophenol produced per minute in each incubation. Also calculate the concentration of substrate in moles per liter for each incubation. Plot alkaline phosphatase activity (p-nitrophenol/minute) on the Y-axis against substrate concentration (moles/liter) on the X-axis. Manually determine V_{max}, calculate one-half that value, and read K_m on the substrate scale (in units of moles/liter).

An alternative method of calculating K_m uses a double-reciprocal (or Lineweaver-Burke) plot, where 1/activity is plotted against 1/substrate concentration. Here, the Y-intercept will be $1/V_{max}$ and the slope of the straight line will be K_m/V_{max}. How does the result of this method to calculate K_m compare with the first method?

For your pH data, simply plot alkaline phosphatase activity (in micromoles p-nitrophenol/minute) vs. pH, and determine the optimum pH for your preparation.

DISCUSSION

Compare your results regarding K_m and optimum pH with those for other enzyme sources. Do you see similarities or differences? How do you account for these results? What can you say about the functioning of alkaline phosphatase in the particular tissue chosen?

ALKALINE PHOSPHATASE IV: PROJECT

INTRODUCTION

In this fourth week of our work on alkaline phosphatase, you have the opportunity to design your own variation on the theme. Using the methods that we have introduced in previous weeks, plan a small experiment designed to answer a very specific question. Perhaps some of your earlier work resulted in less-than-desirable data and so you may wish to repeat an aspect of that work. Or you may wish to pursue a question about alkaline phosphatase that we have not addressed to this point. In thinking up questions for research, a guideline is to make an estimation of what can be both interesting and productive. What experiments are likely to produce interpretable data?

Here are some possibilities:

1. If you have determined pH and substrate effects for alkaline phosphatase from one tissue fraction, you might compare that data with effects found with a different tissue fraction (perhaps one you made or can borrow from a neighbor). Are there different alkaline phosphatases, specific for each tissue or subcellular fraction?

2. You could determine the alkaline phosphatase activity in fresh vs. frozen tissue homogenates. We have a few frogs available for these kinds of experiments.

3. Do other organisms show the same levels or properties of alkaline phosphatase as Xenopus? If you homogenize tissues of other animals (or plants) and test for alkaline phosphatase, do you see similar levels of activity of the enzyme?

4. Can you repeat the results obtained in earlier labs? This is of course one of the major tests of scientific validity and would be a very worthwhile venture!

METHODS AND MATERIALS

Consult previous handouts for detailed methods. As you set up your experiment, which must be done before you arrive in the laboratory for the afternoon, be sure to keep in mind appropriate controls and standards. Ask one question at a time; that is, address the effects of one variable in a given experiment, keeping other conditions constant. Make a chart similar to the ones provided in the handouts to keep your assay tubes organized. You should check briefly with your lab instructor before you start the experiment. He/she may have some suggestions!

RESULTS

Perform the necessary calculations on your data and use spreadsheet or graphics software to plot your results.

DISCUSSION

What conclusions can you make from your experiments? What were some "insecurities" that exist in your data? What additional experiments would you suggest to confirm your conclusions?

BIBLIOGRAPHY

You should find at least three references (primary or secondary) which relate directly to your work on alkaline phosphatase. Make them as specific to your experiment as possible.

ALKALINE PHOSPHATASE V: ANALYSIS OF DATA AND THE REPORT

1. Analysis of data

Your data from Lab 1 (absorbance spectrum and standard curve) should be presented as "XY" or "scatter plots" using graphics software to assist you. A spline curvefit seems to work best for the absorbance spectrum using your average values. The standard curve, however, should be depicted as a linear curvefit. Be sure to calculate the slope of your standard curve as absorbance per micromole.

The data from Lab 2 (fractionation) can be presented either in table form or as a simple bar graph, plotting alkaline phosphatase activity (in micromoles/min) against tissue fraction on the X-axis. You should also calculate the activity of each fraction as a percentage of activity in the homogenate. Does the total of the three fractions equal 100%? Why or why not?

The data from Lab 3 (substrate and pH) will require some analysis. The pH data can simply be plotted as alkaline phosphatase activity on the Y-axis versus pH on the X-axis. What is the "optimum" pH for your fraction? The substrate data, however, should be graphed as alkaline phosphatase activity versus substrate concentration (in moles/liter). Both values will have to be calculated from your original data. The concentration of substrate in the stock solution was 0.01 M. You diluted that concentration by various amounts when you made up your incubation mixtures. For example, if you used 100 ul of substrate + 400 ul of water + 500 ul of buffer + 100 ul of enzyme, you diluted the substrate to 100/1100 of the original concentration, or 1/11 * 0.01 = 0.000909 M.

If you plot alkaline phosphatase activity versus substrate concentration, you should be able to determine a V_{max} value as the highest consistent activity achieved. Calculate one-half that activity, and using your graph, determine the substrate concentration that produces 1/2 V_{max}. This is the K_m value for that substrate-enzyme combination.

Now you should "linearize" your substrate data by calculating the reciprocal of the activity and plotting that against the reciprocal of substrate concentration. (The reciprocal of x = 1/x .) This so-called Lineweaver-Burke plot lets you calculate K_m rather than estimating it graphically. For the Lineweaver-Burke plot, the y-intercept is equal to $1/V_{max}$ and the slope of the line is K_m/V_{max}. Knowing V_{max}

you can of course calculate K_m. How does this calculated value differ from your graphically estimated value? Which is the "best" value, do you think?

For your individual projects, you will have to decide how to present your data, based on the recommendations above. If you are unsure of how to analyze your results and present them in your report, check with one of the laboratory instructors for suggestions.

Wherever possible, compare your results with those of lab colleagues. For example, if you analyzed liver microsomes plus soluble fraction, compare your data with group who analyzed microsomes plus soluble fraction from other tissues.

2. Writing your report

Writing a laboratory report is like writing an original research paper. The usual structure of a research paper is:

Introduction
Methods and Materials
Results
Discussion
Literature Cited

Many research papers also include an abstract which summarizes the paper in 200 words or less. You should write such an abstract after you have written the main body of your report. For guidance in writing these sections, refer to previous handouts which used the conventional structure. Also refer to a published paper in the original literature for additional models from the real world. Examples will be available, or you may select a paper from your own library research as a model. Follow that model consistently as you write your report.

The introduction of the report should explain why the work was done. What were the goals of the research? How does the research help to fill a hole in our knowledge? What were your objectives in doing the work?

The methods and materials section tells how the work was done. What procedures were followed? What research materials were used: the organism, special chemicals, instruments? In the present case, most of the procedures were given in great detail in the handouts. You should not retype these detailed procedures but rather summarize them, and provide details only about changes from the handout and about your individual project.

The results section explains what you found, the data that you generated, explained succinctly in the body of the report and presented as tables or graphs. Each table and graph should be numbered sequentially for easy reference in the text, and each table and graph should be given a brief description (= a "legend") which provides the reader with enough information to know what you did to produce that data. Refer to real scientific papers to see how this is done. WordPerfect will allow you to embed figures in your text, using the "graphics" command. You may wish to try this, or assemble your tables and figures in order at the end of the paper.

The discussion section typically includes your appraisal of what your research means, including its success in meeting the objectives stated in the introduction and its significance in advancing your knowledge of the subject. This is also the place to explain discrepancies or difficulties with experiments, as well as suggestions for future work. If you had initially known what you know now, how might you have changed your experiments? Most importantly, the discussion provides an opportunity to compare your results with those of others. What previous information exists that is relevant to your research? Do your results agree with or complement those previous pieces of information? You should compare your data with that of other investigators in the class. What significance for the life of the organism is suggested by your results?

Science does not operate in a vacuum. And so it is important and necessary to place your work in perspective with the published work of other scientists. The literature cited section formalizes this comparison. Through your library research,

particularly examining the primary reference abstracts on reserve, you should have found previous work that relates specifically to your experiments. These papers should be cited in your report according to an accepted style. Although you must stick to one style throughout your report, the specific style selected may be one of several. For example, some journals ask that you cite references this way (Towle and Holleland, 1987). Others ask you to number your references and place that number in parentheses wherever you cite a reference in the text (1). Footnotes in the tradition of the humanities are <u>not</u> typically used in scientific papers. List each reference once in the literature cited section, and refer to that reference as many times as you need to in the text. Don't use "op cit.", "ibid.", etc.

In the literature cited section itself, the manner of describing the references must be consistent, but several styles are possible here as well. An original article is typically listed as follows:

> Shetlar, R.E., and Towle, D.W. 1989. Electrogenic sodium/proton exchange in membrane vesicles of crab gill. Am. J. Physiol. 256: 123-134.

In this citation, the order is authors, year, title, journal, volume, pages. This order may vary from journal to journal, but you must be consistent within the report.

A typical book chapter would be cited as follows:

> Towle, D.W. 1989. Sodium transport systems in gills. <u>In</u> Comparative Aspects of Sodium-Coupled Transport, R. Kinne, ed. Springer-Verlag, Berlin.

In this citation, the order is authors, year, title of chapter, title of book, editor, publisher, city. Again the order may vary from one journal to another. If in doubt, ask!

LITERATURE CITED

1. Lovett, D.L., C.L Kwasinski, and D.W. Towle. 1989. Ultrastructure and enzyme activity in the gill of the blue crab *Callinectes sapidus*. American Zoologist 29:61A.

2. Towle, D.W. 1991. A research-like experience for introductory biology students. Council on Undergraduate Research Newsletter 12:47-51.

--ERRATA--

Dorothy May
Using the Winogradsky Column to Demonstrate Biodegradation

The article, "Using the Winogradsky Column to Demonstrate Biodegradation" which appeared in BIOSCENE Volume 17(3):December 1991 contained a mistake.

In the <u>Methods</u> section the word "light" was mistakenly changed to "heat." The column needs a <u>light</u> source in the form of a 60-watt bulb to encourage the growth of photosynthetic bacteria.

Coalition For Education in the Life Sciences

In 1991 individuals from thirty life science organizations, representing over 250,000 professional scientists and science educators, called for a coordinated effort of national organizations working together to improve life science education. They established a national life science education network called the Coalition for Education in the Life Sciences (CELS). The mission of the Coalition is to bring the expertise and resources of the life science professional societies to bear upon critical issues relating to life science education in America and to enhance every citizen's knowledge of the complexity and interdependency of living systems from ecosystems to molecules.

The goals of the Coalition are to enhance the ability of the life science community to speak with a unified voice regarding life science education and to promote public understanding of important life science concepts.

The objectives of the Coalition are to develop a framework for life science education that clearly identifies the important principles and concepts that are relevant to every citizen, establish a common language "the Language of Life" among life science disciplines to effectively communicate key ideas to the public, private and government sectors, and increase the role of individual life scientists in 1) influencing the public's image of science and the scientific process, 2) recruiting members of underrepresented groups into the sciences, 3) using alternative teaching and learning methods, and 4) providing teacher enhancement activities.

The activities of the Coalition are guided by a six-member Executive Committee. This Committee comprises educational leaders from a broad and balanced range of life science and science education communities.

CELS's activities are supported by sponsoring organizations and foundations, membership fees, and conferences. CELS offers two membership categories: sponsors and regular members. Both categories offer leadership in the life sciences and linkages to other science, math, and engineering disciplines and the public. Sponsors of CELS contribute $1000 annually and receive appropriate recognition. They are entitled to one complimentary registration for all CELS-sponsored conferences and 10 complimentary copies of all reports. Regular members of CELS contribute $250 annually and are entitled to one complimentary copy of all reports. Both sponsorship and membership of CELS are available to any life science or science education organization that has an interest in life science education.

CELS is a catalyst, a powerful force that coalesces the life science education community in assuming a leadership role which rivals that of the mathematics community. It offers connections to other life science organizations for the purpose of improving life science education and literacy. It provides 1) national visibility of your Society's commitment to life science education and literacy, 2) continued dialogue between your Society, the life science education community and the public, 3) interface between the life science disciplines and other math, science and engineering disciplines, 4) priority listing of your Society's educational activities in the proposed network, and 5) complimentary registration at the upcoming National Life Science Education Conference II and 10 copies of the Conference II Report.

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For more information about the Coalition for Education in the Life Sciences contact:
Amy Chang or Rachel Henry
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(202) 737-3600 or FAX (202) 737-0233

AMCBT AT CELS

The Association of Midwestern College Biology Teachers was represented at both the first and second meeting of the Coalition for Education in the Life Sciences (CELS) which were both held at Wingspread in Racine, Wisconsin, by John R. Jungck as President and then as Past-President of AMCBT. The potential for CELS is great if enough professional biological societies join forces. Unlike chemists who have the American Chemical Society, the physicists and mathematicians who mostly belong to one of two major societies in their respective discipline, the geologists who even the collegiate members belong to and are represented by the National Association of Geology Teachers, we biologists have hundreds of societies none of which can claim even a simple majority of all biologists. Therefore, if we want to have a national voice, we need to select and support some individual or organization with sufficient stature to draw the attention that biologists richly deserve. CELS already has support from three of the largest biological research organizations: AIBS, FASEB, and, in particular, ASM. At this point, NABT and ABLE were not at the second meeting, BSCS was represented by an adjunct staff person (a botanist very committed to collegiate ed cation on leave from a university), thus, AMCBT is in a position to speak for the teaching side at the collegiate level. The option to formally support CELS will be on the agenda at the fall executive commi tee. If you have questions please contact John Jungck at the general fall meeting.

Unifying Science Concepts:

The George Engelmann Mathematics and Science Institute - Summer Science Scholar Program

Today, scientists are probing the stars and cataloging the human genome—explaining the universe and life in ways that previous generations never imagined. Improvements in health care extend life far beyond what was expected a generation ago. From microprocessors to artificial intelligence, technologies growing out of science affect our daily existence in micro-subtle ways as well as in a gross global manner. As we enter a new century, these technologies and their effects will continue to increase at an exponential rate.

As we rapidly approach the 21st century, there is one concern upon which social, economic, and political questions rest: Will there be an adequate supple of trained leaders to answer the new, highly technological challenges facing us? Recent trends have raised doubts about the adequacy of our preparation of future citizens in the fields of science and mathematics. These trends, combined with the past decade's sustained growth in employment opportunities, have led some experts to predict shortages in the sciences and engineering workforce for the future.

Curiosity, in the broad sense of intellect, and competition are two natural components of the human being, and are driving forces for success in every environment including the high-tech society of today. These human behaviors can be altered through the societal mechanism for reward and reinforcement...simply, no reward no achievement. Society can easily direct its focus and the fields of achievement by issuing rewards and encouragement in directions of its choice.

To encourage students to be successful in careers in science, mathematics, and their related areas, we must adjust our reward system so that students will flow into these areas. The cost/benefit ratios must be of such proportions that students will naturally move into technical fields. The reward framework can include both intrinsic and extrinsic mechanisms.

In response to this problem the University of Missouri-St. Louis established the George Engelmann Mathematics & Science Institute in 1988. This intensive, four-week summer experience provides talented high school juniors and seniors with a creative, interdisciplinary approach to learning advanced concepts in science. In each of the past four summers, fifty St. Louis-area high school students have participated in the Engelmann Institute. The Institute is a highly successful program that provides the structure for developing intrinsic values for curiosity and academic achievement.

A focal point of the Engelmann Institute is the Science Seminar Series. This group of lectures addresses related subject matter in the interdependent disciplines of biology, chemistry, physics, and mathematics. The program introduces Engelmann participants to a basic set of concepts and then helps them apply that information to relevant, current problems illustrating paradigms presented in Philosophy of Science. Like a mental microscope, the program focuses on such specifics as the AIDS virus, genetic engineering, and the problems of world overpopulation. Unlike the mechanical approach associated with operating a microscope, these topics are approached from the perspective of the philosophy of science and the pragmatic definitions of truth.

Participants take field trips to research-based corporations and institutions to observe the applied component of the Science Seminar Series and to help reinforce career opportunities. These young people not only exercise their minds for four weeks in the middle of the summer, they also exercise their bodies on the softball field and the volleyball

court, where they are introduced to the physiology and physics of the sports. The concluding days of the program are filled with finalizing research papers and making oral presentations.

The George Engelmann Mathematics and Science Institute is a successful program that provides talented high school students with a creative, interdisciplinary approach to learning advanced concepts in mathematics and science.

Additonal information regarding The George Englemann Mathematics & Science Institute and Summer Science Scholar Program can be obtained by contacting:

Dr. Charles R. Granger, Director
The George Engelmann Mathematics & Science Institue: UM - St. Louis
8001 Natural Bridge Road
St. Louis, Missouri 63121-4499
(314) 553 - 6226

RESEARCH AND SCHOLARLY WORK IN CHEMICAL EDUCATION

Reprinted from the Division of Chemical Education of the American Chemical Society

The following list suggests activities that might be used in making tenure and promotion decisions for faculty members whose emphasis is in the area of chemical education. In addition to excellence in teaching, research, and service (the traditional criteria for granting tenure or promotion), activities such as these should also qualify as scholarly work in chemical education.

1. Development of New Courses and/or Curricula, such as:
   - Chemistry Courses for Science or Non-Science Majors
   - Science Education courses for Teachers
   - Laboratory Courses
   - Special Topics Courses

2. Leadership at Professional Meetings (Local, Regional, National, or International)
   - Invited Lecturer
   - Presenter of Papers or Posters
   - Orgainzer of Symposium or Workshop
   - Chairman of Professional Organization or Committee

3. Published Articles in Journals such as the following:
   - Journal of Chemical Education
   - Journal of College Science Teaching
   - Science Education
   - School Science and Mathematics
   - Journal of Research in Science Teaching

4. Submission and Funding of Grant Proposals for projects such as:
   - Chemical Education Research
   - Laboratory Instrumentation
   - Teacher Pre-Service or In-Service Programs
   - Science Programs for Primary or Secondary Students
   - Production of Chemistry Teaching Materials

5. Contributions Toward Instructional Improvement, such as:
   - Introducing New Chemical Demonstrations
   - Developing New Laboratory Experiments
   - Providing Guidance for Teaching Assistants
   - Creating Models or Other Visual Aids for the Classroom
   - Finding New Uses for Computers in Chemistry Teaching
   - Developing Audio-Visual Materials or Computer Software

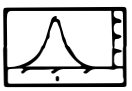
6. Other Activities in Chemical Education:
   - Authoring a Textbook or Support Materials
   - Review of Textbook or Journal Manuscripts
   - Review of Chemistry Programs or Grant Proposals
   - Interpretation of Chemistry for the Public
   - Involvement in Writing of National Chemistry Exams
   - Participation in Chemical Meetings or Workshops
   - Service on Chemical Education Committees or Task Forces

The BioQUEST Collection

Edited by John R. Jungck
Co-Editors: John N. Calley, Nils Peterson & Jim Stewart
Managing Editor : Patti Soderberg

The new BioQUEST software collection is expected to be published early this summer on a CD-ROM; a compact disk that stores information in digital form. The first compact disk, an examination copy, is expected to cost under $100. When a publication date is selected, The ASDG of the University of Maryland will announce the final price and details about site licenses.

For More information contact; Academic Software Developement Group, University of Maryland, College Park, MD 20742 (ASDG@UMDD.BITNET)

CURRICULUM MODULES	CONSORTIUM AUTHORS
Introduction to the Three P's Chapter - Practical Introduction & Philosophy	John R. Jungck, Beloit College John Calley, University of Arizona Jim Stewart, University of Wisconsin
Introduction to BioQUEST Hypercard Tour	Nils Peterson, From The Heart Software
 **Biometrics** Chapter and Hypercard Stack	Daniel Hornbach, Macalester College
 **Biota** Software - Predator / Prey relationships	Jim Danbury, Ben Jones, John Kruper Jim Lichtenstein, Eric Nelson, Jeff Schank Bill Sterner, Joyce Weil, Bill Wimsatt; University of Chicago
 **CVCK** Software Cardiovascular Construction Kit	Sarah A. Douglas, Univ of Oregon Nils Peterson, From the Heart Software Daniel Udovic, Univ. of Oregon
Data Collection and Organization Chapter and the "Bumpus" data set	Frank Price, Hamilton College
 **Environmental Decision Making** Software - Systems Ecology	H.T. Odum, Univ. of FL Gainesville E.C. Odum, Santa Fe Comm. College Nils Peterson, From The Heart Software
 **Genetics Construction Kit** Software-Mendelian Genetics	John Calley, Univ. of Arizona John R. Jungck, Beloit College
 **Modeling** Chapter-Modeling in Biology	William Wimsatt, Univ. of Chicago Jeff Schank, Univ. of Chicago
 **Sequence It!** Software - Protein sequencing	Alan Place, Univ. of Maryland Thomas Schmidt, Univ. of Maryland

Application For Membership

ASSOCIATION OF MIDWESTERN COLLEGE BIOLOGY TEACHERS

NAME:_____ DATE:_____

TITLE:_____

DEPARTMENT:_____

INSTITUTION:_____

STREET ADDRESS:_____

CITY:_____STATE:_____

ZIP CODE:_____

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WORK PHONE:_____ FAX NUMBER:_____

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MAJOR INTERESTS:

( ) 1. Biology
( ) 2. Botany
( ) 3. Zoology
( ) 4. Microbiology
( ) 5. Pre-professional
( ) 6. Teacher Education
( ) 7. Other

RESOURCE AREAS:

_____

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RESEARCH AREAS:

_____

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Have you been a member before?_____ If so, when?_____

PLEASE MAIL

MEMBERSHIP APPLICATION

FORMS TO:

Edward S. Kos
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CURRENT DUES ARE **$25.00**