

## Introduction to Nucleic Acids

### Definitions

By definition, nucleic acids are biomolecules that store genetic information in cells or that transfer this information from old cells to new cells.

There are two groups of nucleic acids: DeoxyRiboNucleic Acid (DNA) and RiboNucleic Acid (RNA).

DNA codes for the functioning of the cell. DNA is located mainly in the nucleus of the cell (with a small amount in the mitochondrion of eukaryotic cells -- to be discussed at a later date); DNA is double stranded.

RNA is the "worker" that helps get the DNA message out to the rest of the cell. RNA is primarily in the cytosol of the cell. RNA is single stranded – with one "exception": t-RNA has 4 regions of double stranded nucleotides that follow no rules that we know of.

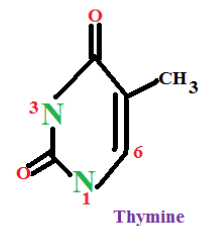
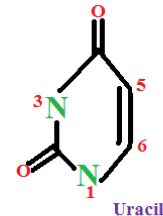
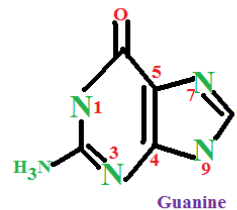
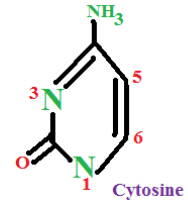
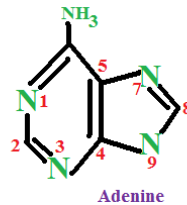
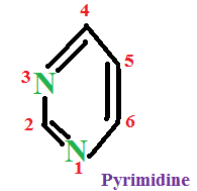
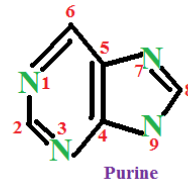
BOTH nucleic acids are "codes" for the cell and, hence, the body's activities at the cellular level.

Nucleic acids consist of nitrogenous compounds called purines or pyrimidines, a carbohydrate and phosphate. The figure shows what the structures of purine and pyrimidine look like in the lower right-hand corner.

Purine is a 2-fused ring system that consists of one 6-membered ring fused to a 5-membered ring. In each ring, there are 2 nitrogens. Pyrimidine has only the 6-membered ring with the 2 nitrogens (image, above).

### Purines

The two purines of significance are adenine and guanine (image top of page). Note that they differ very simply: adenine has an amino group on the top of the 6-membered ring, while guanine has an amino group between the 2 nitrogens on the 6-membered ring, a double bonded oxygen in place of adenine's amino group (called a ketone) and has lost a double bond to accommodate the ketone.



## Pyrimidines

There are three pyrimidines of interest in Biology: Cytosine, uracil and thymine (image, top of previous page). Cytosine has a ketone between its two nitrogens, an amino group atop its ring and has lost a double bond to accommodate the ketone. Uracil is a modified form of cytosine, where the amino group is replaced by a ketone and a double bond in the ring is lost to accommodate the new ketone. Thymine is uracil with a CH<sub>3</sub> (methyl) group attached adjacent to the ketone at the top of the ring.

Generally, the 5 nitrogenous compounds with their carbohydrate moiety are abbreviated as one letter abbreviations: A, G, C, U, T. It is important to keep in mind that when referring to RNA, these shortcuts are o.k.

When referring to them in DNA, it is important to remember that we're discussing dA, dG, dC, dU, dT. The reason for this is shown in the figure at right. (d-R)

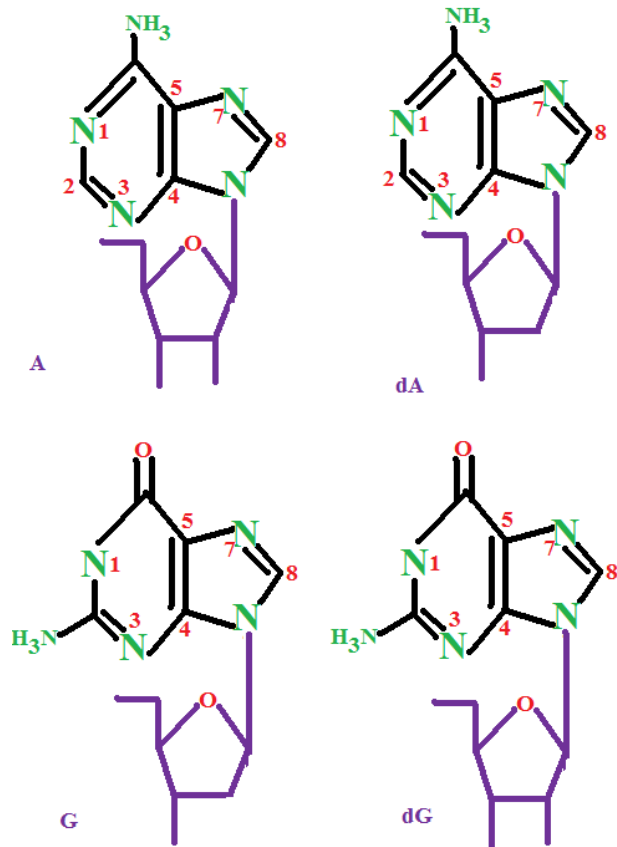
The carbohydrate that binds with the nitrogenous compounds in RNA is ribose. The carbohydrate that binds with the nitrogenous compounds in DNA is deoxy-ribose. Deoxyribose is ribose that has had the 2' -OH group removed (see arrow on figure).

The last portion of nucleic acids is the phosphate group. This group is of immense importance, as it is through this group that DNA and RNA are "held together".

## NucleoSides

In the hierarchy of nucleic acid structure, there are two more levels of nomenclature: nucleoSides and nucleoTides.

We'll address the nucleoSides first. Nucleosides consist of a purine or pyrimidine and a carbohydrate. When a purine or a pyrimidine reacts with ribose or deoxy-ribose, water is always one of the products.



When the purine is adenine and it reacts with ribose, the other product is adenosine (A). When adenine reacts with deoxy-ribose, the other nucleoside is deoxy-adenosine (d-adenosine or dA) (top, previous page).

Likewise with the remainder purines and pyrimidines.

One point to keep in mind is that d-uridine and thymidine are produced only in the lab, not in DNA or RNA, respectively.

When the purine is guanine and it reacts with ribose, the other product is guanosine (G). When guanine reacts with deoxy-ribose, the other nucleoside is deoxy-guanosine (d-guanosine or dG) (top, previous page).

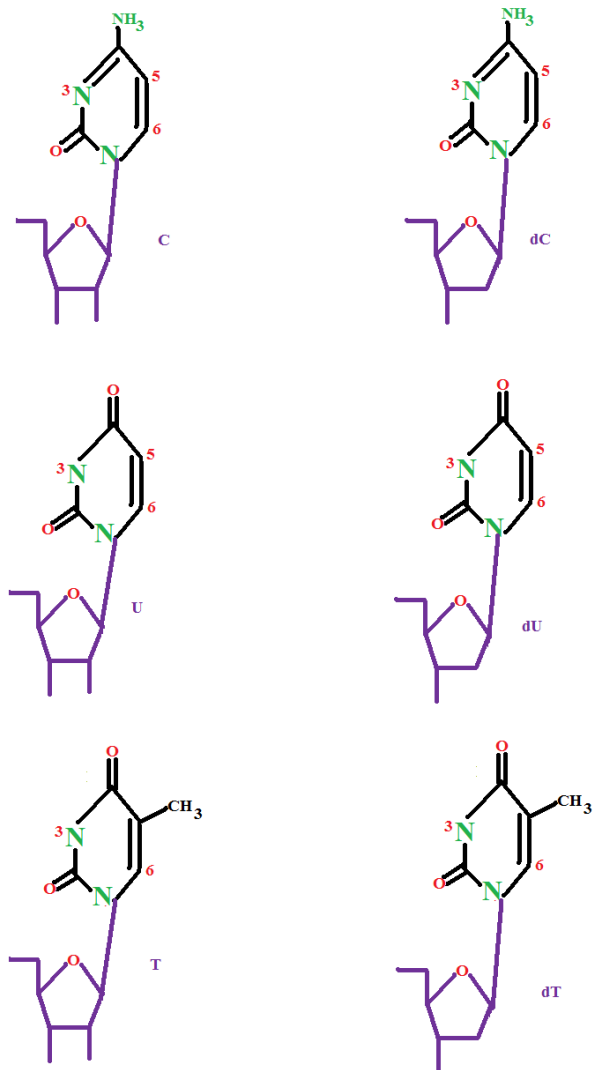
When the pyrimidine is cytosine and it reacts with ribose, the other product is cytidine (C). When cytosine reacts with deoxy-ribose, the other nucleoside is deoxy-cytidine (d-cytidine or dC).

When the pyrimidine is uracil and it reacts with ribose, the other product is uridine (U). When uracil reacts with deoxy-ribose, the other nucleoside is deoxy-uridine (d-uridine or dU). One point to keep in mind is that d-uridine and thymidine are produced only in the lab, not in DNA or RNA, respectively.

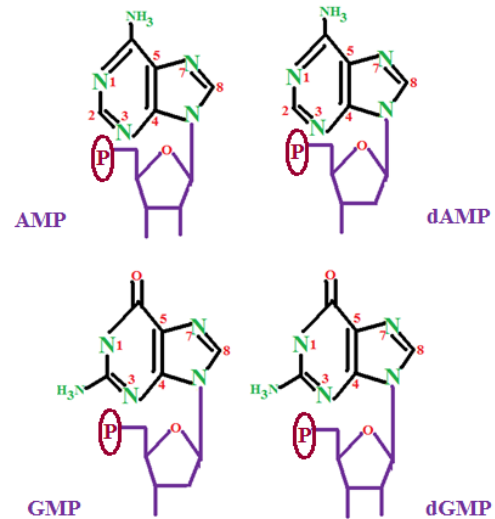
When the pyrimidine is thymine and it reacts with ribose, the other product is thymidine (T). When thymine reacts with deoxy-ribose, the other nucleoside is deoxy-thymidine (d-thymidine or dT). One point to keep in mind is that d-uridine and thymidine are produced only in the lab, not in DNA or RNA, respectively. All pyrimidines are shown at lower right.

### NucleoTides

The last simple level of nucleic acid nomenclature hierarchy is the nucleoTide. Nucleotides are nucleosides that have



added a phosphate group to the 5' carbon of ribose or deoxy-ribose (figures at right, this page). Biochemist's short-hand the  $\text{PO}_4^{-3}$  as a "P" with a circle around it (Ⓟ). Note that if there were three (3) phosphates instead of one, the names would end as "triphosphate", hence, ATP is adenosine triphosphate and dATP is deoxy-adenosine triphosphate.



### Primary Structure of Nucleic Acids

Linked by phosphodiester bonds between [d] ribose (see following page, top center; note "arrows for anti-parallel strand running). GC and AT are always in DNA (dGdC dAdT DNA) and pair up as written when the secondary structure of DNA forms.

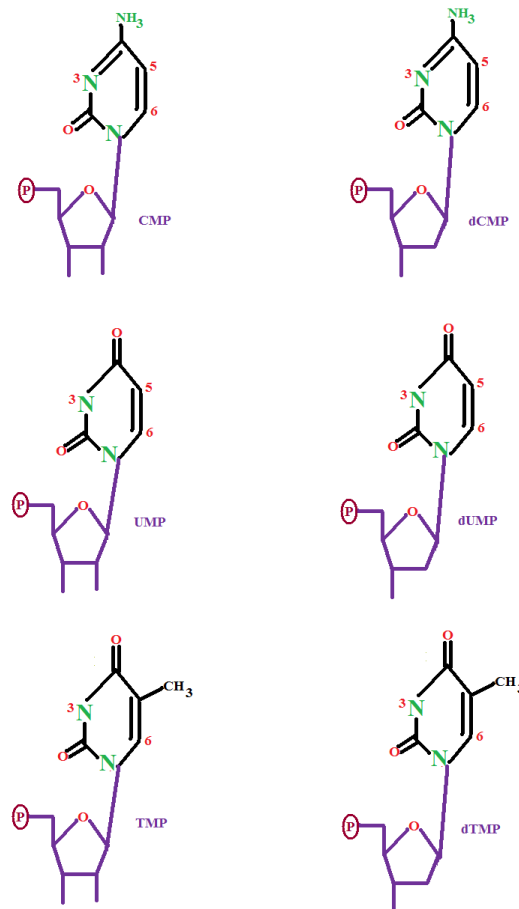
### RNA – Primary Structure

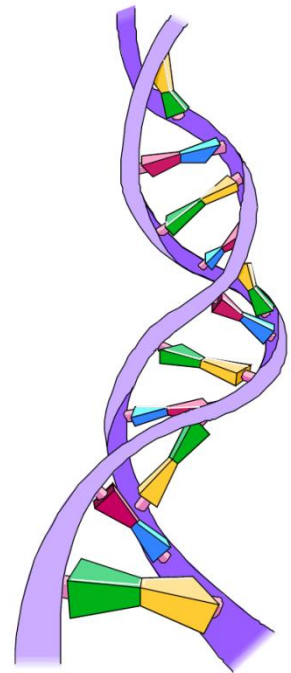
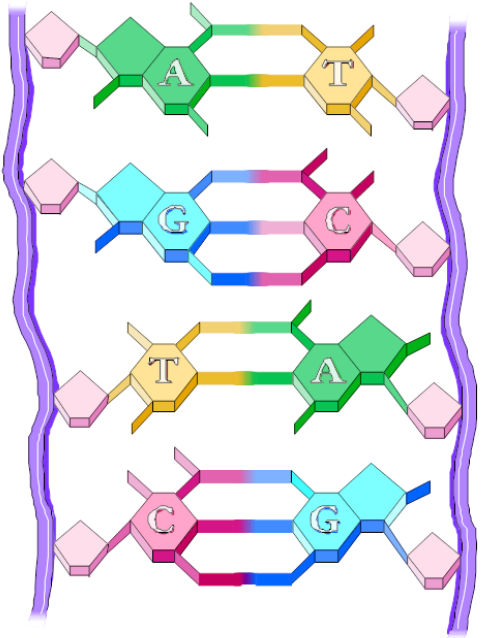
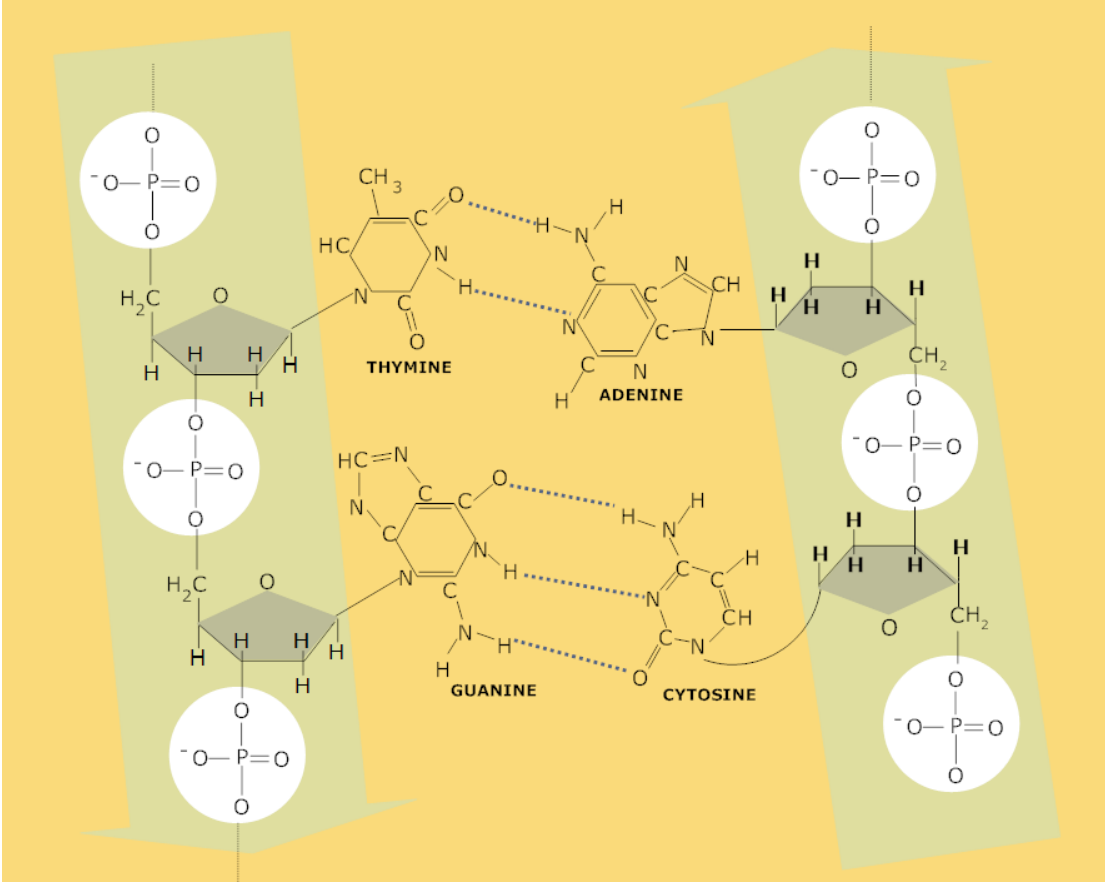
The primary sequence of RNA is also linked by phosphodiester bonds in the same manner as DNA. Difference is that U is substituted for T in RNA. GC AU RNA.

### Secondary Structure of DNA

There are two linear strands of DNA (bottom left, following page) coiled in a right handed double helix (bottom right, next page). These strands run in ANTI-parallel directions (see image at top center next page; note arrows, again). One strand runs 3' to 5' and the other runs 5' to 3' so that the strands are always complimentary to each other.

The sugar phosphate backbone is on the OUTSIDE of the helix. The nitrogenous bases are on the INSIDE of the helix. The OUTSIDE of the helix is HYDROPHILIC; the inside of the helix is HYDROPHOBIC. The bases are almost perpendicular to helical direction.

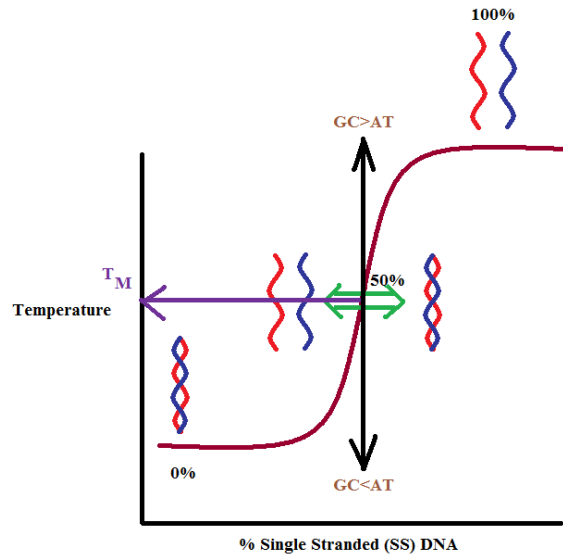




## Physical Properties of DNA

The physical properties of DNA depend upon, for the most part, the secondary structure of the DNA. These properties are all dependent upon H bonds!

Melting curves take advantage of the hydrogen bonds in DNA (image at right). At the left end is 100% double stranded DNA; at the right end is 100% single stranded DNA. In a manner very similarly to the first derivative curve determinations for amino acid pK value, at the mid-point of the melting curve, there is a 50/50 mix of DS and SS DNA. This point is called the melting temperature ( $T_M$ ).



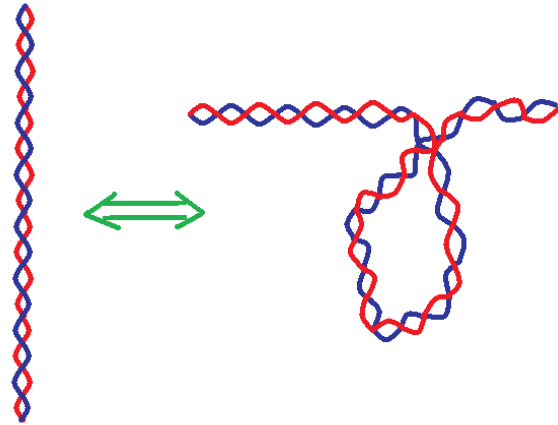
The melting temperature is impacted by the GC and AT content: If there is more GC, the  $T_M$  increases; conversely, if there is less GC, the  $T_M$  drops. This is due to the number of hydrogen bonds: GC takes three to form the double strand and AT takes two hydrogen bonds – simply pure energy differences.

Other Physical Property Changes with Denaturation			
$A_{260}$	↓	↑	↑↑↑
Purines unstacking and opening up for greater absorption at 260 nm (UV region of spectrum).			
Viscosity	↑	↓	↓↓↓
The less organized the DNA, the faster it flows (remember your viscometry experiment in CHEM 121?).			
Buoyant Density	↑	↓	↓↓↓
The less organized the DNA, the less dense it is and it “floats” differently in a concentration gradient.			

1° & 2° Structures do NOT Account for All the Physical Properties in DNA. What else is there???? 3° (tertiary) and 4° (quaternary) structures!

### 3° Structure

Functionally, tertiary structure takes the standard double helix and puts supercoils into it – much like twisting a rubberband to its twisting point, then twisting the twisted rubberband on itself, image at right.



### 4° Structure

The quaternary structure depends upon the binding of a special class of proteins called histones. These histones are laden with arginine and lysine residues, making them positively charged. These positively charged R groups bind with the phosphate on the DNA molecules to “force” the molecule to further compress.

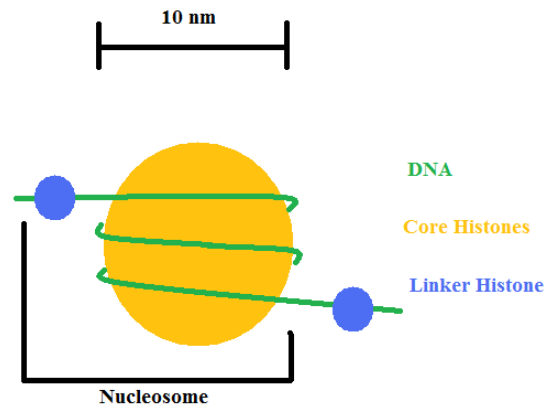
Depends on 5 classes of positively charged proteins called histones.		
Name	Quantity	Type
H1	1	Linker
H2A	2	Core
H2B	2	Core
H3	2	Core
H4	2	Core

### DNA “Compression” – 1

In this stage of compression, a strand of DNA that is 30 nm in length forms into tertiary structure. This is called a “30 nm fiber” of DNA.

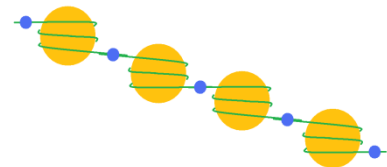
## DNA "Compression" – 2

The second stage is for the DNA to interact with histones (8 in the core and 1 outside; see table above on previous page) linking the molecules into a "bead on a string" model (image at right).



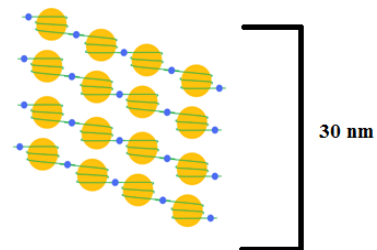
## DNA "Compression" – 3

The bead on a string fiber of DNA elongates (image middle right), then begins to compress, further, into another "30 nm fiber" (image, bottom right).



## DNA "Compression" – 4

The new 30 nm fiber continues compressing until an actual chromosome "appears". The structure of the chromosome is not only by way of quaternary structure, but super-secondary structure, as well, i.e., more twisting and coiling of the genetic material after the histones have organized it. A chromosome is 1/46 of chromosomes in nucleus of cell.

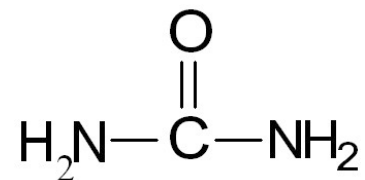


Experimental

## Colorimetric Determination of Blood Urea Nitrogen (BUN)

### Introduction

Urea is the final product of the urea cycle. Urea is the non-toxic, excretory form of ammonia in the body. Urea is synthesized from ammonia and carbon dioxide. The source of the carbon dioxide is the TCA (Krebs' Cycle), whilst the ammonia source is amino acid catabolism, as well as pyrimidine catabolism. Urea is primarily excreted in urine, although a small amount is excreted in sweat. Urea's structure is illustrated, at right:



BUN is used clinically to determine or follow various disease states (table, below), as well as to determine the extent of the disease state, i.e., how progressed the disease is.



BUN Applications	
Increased Values	Decreased Values
Hyperthyroidism	Acromegaly
Myocardial Infarction	Hepatitis
(Congestive) Heart Failure	Late Pregnancy
Decreased Renal Function	Dehydration
Bowel Hemorrhage	

BUN's performed in the clinical laboratory are determined by that lab's processing instrument - many changes have occurred in the last 20 years in instrumentation. BUN's performed in teaching, research or field/combat hospital laboratories are performed by primitive methods, relatively speaking, that utilize standard curve preparation to determine the concentration of analyte in blood (the BUN in this instance).

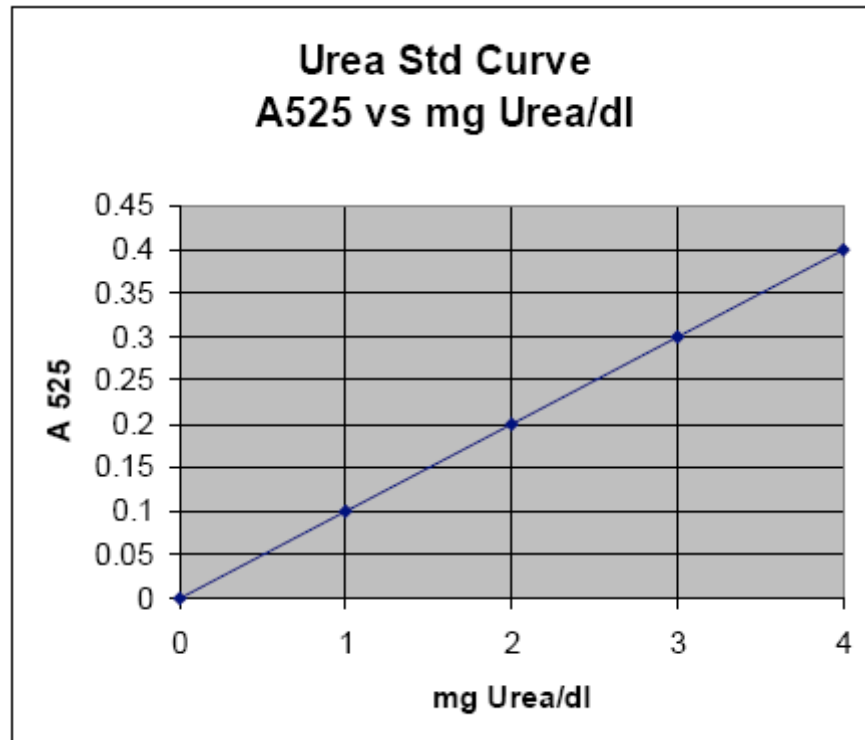
A standard curve is a plot of the absorbance at a specific wavelength (in nm) of light by a colored compound on the "Y" axis versus the concentration of analyte on the "X" axis. The absorbance is usually represented in the following form:  $A_{\text{###}}$ , where the "A" stands for Absorbance and the "###" stands for the wavelength in nm, e.g.,  $A_{525}$ , is the absorbance at 525 nm. The analyte is detected as a "colored compound" which is a reaction product between a dye-like molecule and the actual analyte (urea in this case).

To make a standard curve, one must have "standard solutions" prepared to run alongside the unknown samples (a patient's sample, for example). While it is beyond the scope of this experiment to determine what standards need to be prepared, suffice it to say that research labs eat up a lot of time figuring this out when developing a new assay.

Let's take some sample data and build a standard curve and apply that curve to an assay. The table, below summarizes standard and unknown sample data:

$A_{525}$	Concentration of Urea (mg/dl)
0	0
0.1	1
0.2	2
0.3	3
0.4	4
0.25	Unknown Sample

The standard data would plot as shown below:



Never, never, never plot your unknown/patient's samples with the standard data – you will use the standard data to determine the unknown/patient's sample values. All samples, standards AND unknowns, are always run at the same time, i.e., under the same, IDENTICAL, reaction conditions and are read in the same instrument (spectrophotometer in this experiment).

Our unknown sample had an  $A_{525}$  of 0.25 (table, above). How do we figure out how much urea (BUN) is present in this sample?

Step 1: Find the 0.25 absorbance ("Y" axis) on your standard curve (above). Mark it.

Step 2: Place a ruler so that you can find where the 0.25 reading crosses the standard curve (the straight line from your data points).

Step 3: Mark that point on the curve.

Step 4: Turn your ruler 90 degrees so that it is now perpendicular to the concentration axis (X" axis).

Step 5: Align the point you marked on the standard curve perpendicular to the "X" axis.

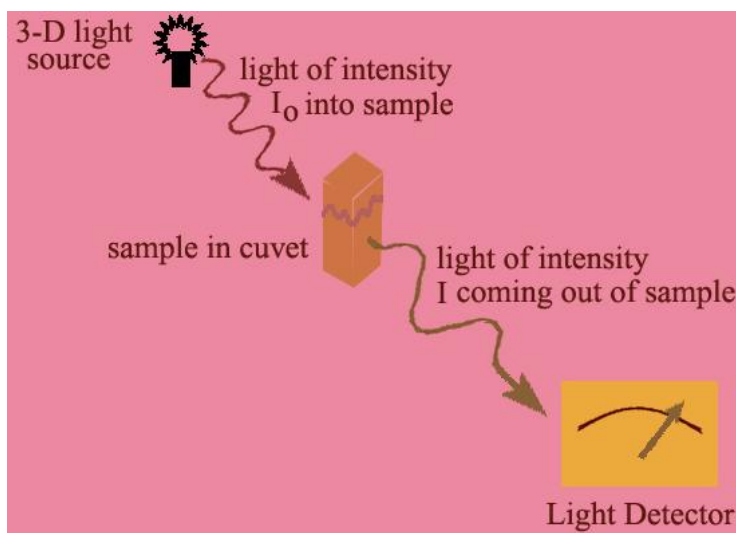
Step 6: Mark that point on the "X" axis.

Step 7: Read where you marked it for the value. In this example, an  $A_{525}$  of 0.25 corresponds to 2.5 mg Urea/dl, which is the concentration of your unknown sample.

Did you notice 1) that the origin on this curve is (0,0) and 2) that the line is straight? The concentration of analyte is directly proportional to the amount of light absorbed by the colored product (to a limit). This statement describes the Beer-Bougier-Lambert Law:  $A = abc$ , where  $A$  = the absorbance at some wavelength of light,  $a$  = the molar absorptivity of the compound, which is a constant,  $b$  = the path length of light through the sample holder (a cuvet) which equals 1 cm and  $c$  = the molar concentration of the analyte.

Did you notice that this equation is in the form of a straight line? And did you notice that the y intercept is at zero? Good! Now you know why the origin is at (0,0) and why the line is straight!

What about this thing called a spectrophotometer? For all intents and purposes a spectrophotometer consists of a light source (adjustable to wavelength), a light path, cuvet (sample) holder and a detector (image at right):



For our purposes (and you can learn more about this in CHEM 122) and/or in Analytical Chemistry and/or in Instrumental Analysis), the darker a sample, the less light that is transmitted through the sample (of intensity  $I$  in the graphic, above) and, hence the more light that is ABSORBED by the sample.

Note that the relationship in the Beer-Bougier-Lambert Law indicates that as you increase standard concentrations, the absorbance increases proportionally – a key point to figuring out your data. Also note that since the relationship is linear, when you plot your data, make sure you start at the origin and that you fit your best straight line through your data points.

## Materials and Methods

### Materials

The table, below, summarizes the supplies you'll need for this experiment:

Reagent A	Standards	Disposable pipets
Reagent B	Unknown sample	Syringe with latex tubing
Distilled water	Boiling water bath	Spectrophotometer
Disposable test tubes	Timer/clock	Beaker tongs
Cuvets	Test tube rack	Sink

**Note to INSTRUCTOR:** Stock urea standards are already prepared. They require dilution for use. The following tables show the sequence of dilutions to follow – do not have students do this.

Table of Stock Solutions		
Standard Number	Grams urea	mL water
1	0.15	100
2	0.30	100
3	0.45	100
4	0.60	100
5	0.75	100

To get to the working standards, follow these steps:

#### **FIRST DILUTION:**

Set up 5 test tubes – one for each stock solution. Into each test tube, pipet 9 drops of distilled water. In test tube #1, pipet 1 drop of standard #1; into test tube #2, pipet 1 drop of standard #2; into test tube #3, pipet 1 drop of standard #3; into test tube #4, pipet 1 drop of standard #4; into test tube #5, pipet 1 drop of standard #5. Vortex well.

#### **SECOND DILUTION:**

Set up 5 more tubes – one for each of the previously diluted samples. Into each test tube, pipet 3 drops of distilled water. In test tube #1, pipet 2 drops of standard #1; into test tube #2, pipet 2 drops of standard #2; into test tube #3, pipet 2 drops of standard #3; into test tube #4, pipet 2 drops of standard #4; into test tube #5, pipet 2 drops of standard #5. Vortex well.

You now have enough diluted working standards for 4 students – 8, if they work in pairs. If you have more than 8 students, adjust the dilutions proportionally. Dilute the unknown stock solution identically as the stock solutions, remembering that each student or pair of students needs at least 4 drops of this sample to run in triplicate (the extra drop is to account for whatever they lose on the walls of the test tubes and pipets).

## Methods

Set up your boiling water bath and get it going. Obtain 9 disposable test tubes, standard working solutions and unknown sample (this one will be run in triplicate). Obtain 6 mL Reagent A and 4 mL Reagent B – mix together.

Using the table, below, set up your tubes and dispense water, sample, unknown and reagents is indicated (where dashes “–” are placed, do not add that reagent to that tube; read the table DOWN for tubes and ACROSS for reagents to add to that tube):

Tube	1	2	3	4	5	6	7	8	9
mg/dl	0	15	30	45	60	75	??	??	??
gtt water	1	--	--	--	--	--	--	--	--
gtt Std	--	1	1	1	1	1	--	--	--
gtt unknown	--	--	--	--	--	--	1	1	1
mL Rx mix	1	1	1	1	1	1	1	1	1

Vortex all samples and place them in your boiling water bath for EXACTLY 10 minutes. After 10 minutes, remove the tubes and put them in a beaker of running cold water at the sink for 5 minutes. After 5 minutes, pour each sample into its own cuvet (keep track of the cuvetts and hold the cuvetts properly so that your oils from your fingers won't interfere with the light absorption). Take your samples to the Spectrophotometer.

To use the Beckman DU-64 Spectrophotometer, follow these steps:

**1) Turn on the master switch at the surge protector (this will be done by your instructor at the beginning of lab).**

**2) Press the “VIS” key.**

**3) Allow a short warm-up period.**

**4) Press the “ABS” key (for Absorbance).**

**5) Press “5”, “2”, “5” and the “λ” key (sets it at 525 nm).**

**6) Place your “0” standard in a cuvet (from test tube #1) and**

**7) Press the “CALB” button (blanks out the reagents – BTW: the blank will be yellow and your samples will be shades of pink to fuchsia – that’s how it’s supposed to be).**

**8) Remove the “0” standard.**

**9) Insert the next standard (from test tube #2).**

**10) Press the “READ” button.**

**11) Record the result in the data sheet, below.**

**12) Remove that sample.**

**13) Repeat steps 9-12 for the rest of your standards and unknown samples until you’re done.**

**14) The last person to use the spectrophotometer please turn it off at the surge protector after the last sample has been analyzed.**

Record your data in the spaces, below:

Sample (mg Urea/dl)	$A_{525}$
1 (0)	
2 (15)	
3 (30)	
4 (45)	
5 (60)	
6 (75)	
7 (?)	
8 (?)	
9 (?)	

Once you have completed your data collection and have cleaned up your work bench, using your computer and Excel or some such software, plot your data to generate a standard curve and determine the values of your unknown samples (and the average value of the unknown).

Based upon your standard curve, record the values for your unknown sample:

Sample 7	Sample 8	Sample 9
mg/dl	mg/dl	mg/dl
AVERAGE VALUE	mg/dl	