INTRODUCTION
Casein is the main protein found in milk. Proteins are biopolymers, large molecules with molecular masses ranging from 5000 up to several million daltons. Proteins are constructed from 20 monomers. The monomers are called amino acids. Amino acids have the following generic structure:

R represents any one of the 20 groups which, when attached to the middle carbon, makes one amino acid differ from another. If two amino acids, alanine and serine, reacted with one another, they could do so as shown in the following diagram:

The dotted ellipse highlights the OH from the acid group of alanine, and the H from the amine group of serine. A water molecule forms as the OH and H react with each other. The C and the N then bond to each other, joining the two molecules together. The resulting compound is called a dipeptide. The molecules could also react in the reverse order: the acid group on serine reacting with the amine group on alanine. There are two dipeptides possible with these two amino acids. Since there are 20 different amino acids, there are innumerable possibilities for the construction of protein molecules. Each one in living systems is tailor made, however, and the order and number of the amino acids are precisely controlled.

In the dipeptide shown, notice that the attached group on the alanine is nonpolar, while the attached group on the serine is polar. Casein has a large number of polar side groups, which makes it soluble in water solutions. In addition, many of the serine side chains in casein form ester bonds with phosphoric acid:

The phosphoric acid groups, with their double negative charge, attach to Ca^{++} ions. Casein has other side groups on it that also exist in a negatively charged state. If an acid is added to milk, hydrogen ions from the acid attach to the negatively charged groups, and the casein becomes insoluble, precipitating out of the milk.

Casein has been used as a glue since the days of ancient Egypt, Greece, Rome, and China. It is mixed with sodium carbonate and calcium hydroxide, and then dissolved in water. The function of the sodium carbonate is to react with calcium hydroxide to produce sodium hydroxide:

The sodium hydroxide reacts with casein, producing sodium caseinate, which is sufficiently water soluble to form a well dispersed sol, a solid dispersed in a liquid:

When the sol is spread on the surface of objects to be glued, the sol wets the surfaces and adheres to them. When the water either evaporates, or soaks into the objects, the solid protein molecule remains, adhering to both objects.

In today’s experiment, you will separate casein from milk, and you will use dried casein to prepare glue.
**EXPERIMENT**

Supplies:
- From the Cart:
  - 1 cheesecloth
  - 1 filter paper
  - 1 Büchner funnel and filter flask
  - 1 mortar and pestle
  - 1 2 x 3” ziplock bag
  - 1 baggie
  - 2 small pieces of wood
- from your drawer:
  - 1-100 ml graduated cylinder
  - 1-600 ml beaker
  - 1-250 ml flask
  - 1 thermometer
  - 1-250 ml beaker
- 50 ml milk

**Part 1: Separation of Casein from Milk**

Set up an apparatus as follows:

![Apparatus Diagram]

Use the graduated cylinder to obtain 50 ml of milk. Pour it into the flask. Watch the temperature of the milk carefully. When it reaches 40°C, turn off the burner, and raise the flask out of the hot water bath. Add 20 drops of concentrated acetic acid to the milk while stirring constantly with a stirring rod. Do not stir with the thermometer. The reason for not stirring with the thermometer is that it is expensive, and stirring runs the risk of breaking the thermometer. The casein should flocculate, that is, precipitate into loose clumps. Hold the cheesecloth tautly over the 250 ml beaker, and pour the contents of the flask through the cheesecloth. Dump the liquid into the sink, dry the beaker, and then transfer the glob from the cheesecloth into the beaker. To remove water from the casein, you will do an ethanol extraction. Pour 25 ml of ethanol into the beaker. Use your scoopula and the micro spatula to divide the casein into as fine a slurry of solid in liquid as your patience will allow. Pour off the ethanol into the sink. Add 25 ml more of ethanol, and continue to mix and divide the solid as finely as possible. Transfer the solid/liquid mix into a connected Büchner funnel (see the Büchner Funnel discussion on page 99). Allow the drained solid to remain in the funnel for a few minutes to help it dry. Transfer the solid to a few thicknesses of paper towel, and go on with Part 2 before you weigh the casein. This gives some drying time. After weighing, transfer the casein to a baggie, and take it home as a trophy. You will not use this casein in Part 2.

**Part 2: Casein Glue.**

Dry casein is required here, so you will not be able to use the casein you just collected. Weigh out 2.0 gram of dried casein (dried skim milk), 0.6 gram of calcium hydroxide, and 0.3 gram of sodium carbonate. Transfer the solids to a mortar. Use a pestle to titurate (grind finely) the solids. Dump the contents of the mortar onto a creased piece of paper, and then transfer into a ziplock bag. Mark the bag: “Dry Casein Glue”. Take a small portion of the dry mix, and place it on a watch glass. Add enough water to make a paste. Mix the water and powder with a stirring rod to remove any lumps. Use the rubber policeman on a stirring rod to apply the glue to two pieces of wood. Apply pressure to the glued objects with a clamp or with books. Let the wood sit at least overnight, then check to see how well the glue binds. Use the rest of the dry glue mix at home.

**Data:**

**Part 1:**
- Grams of casein _______ g
- Appearance of casein: __________

**Part 2:**
- Appearance of dry glue mix:
- Consistency of wet mix:
- Smell of wet mix:
QUESTION

1. Here are three amino acids. There are six different tripeptides that can be formed, each using all three of these amino acids. Show the six tripeptides. Use the dipeptide on page 1 as a template, but note that these six structures will be tripeptides.

\[ \text{Cysteine} \]

\[ \text{Phenylalanine} \]

\[ \text{Glutamine} \]

2. The number of polypeptides that can be formed from \( n \) amino acids is \( n! \), \( n \) factorial, which is \( 1 \cdot 2 \cdot 3 \cdot \ldots \cdot n \). How many polypeptides could be formed from 4 amino acids? From 5? From 6? Use the factorial button on your calculator to see how many polypeptides could be made from the 20 natural amino acids.