CREATING COACERVATES
Adapted by Larry Flammer

SYNOPSIS
Students mix a carbohydrate solution with a protein solution, adjust the pH, and view coacervates: amoeba-like objects, which change shape, flow, merge, divide, form "vacuoles", release "vacuole contents", and show other life-like properties.

MAIN CONCEPTS
Under suitable conditions, life-like structures can form naturally from relatively simple materials.
The origin of life on earth did not involve supernatural processes

ASSESSIBLE OBJECTIVES
1. recognize coacervates
2. realize that complex life-like cell-like structures can be produced naturally from simple materials with simple changes.

MATERIALS
1. safety goggles & lab aprons
2. compound microscope, slides, coverslips
3. test tube rack with clean small culture tubes (13x100 mm works well)... one tube per student.
4. one medicine dropper per tube
5. one dropping bottle with 0.1M HCl solution
6. pH paper in dispenser with color code
7. one 50-ml beaker with coacervate mix (5 parts of 1% gelatin soln + 3 parts 1% gum arabic soln.). You can make the 1% solns day before lab, (adding a pinch of mold inhibitor seems to help). Mix the two solutions (5:3 ratio) day of lab, then dispense into little beakers. For 5 classes, 500 ml of gelatin solution and 300 ml of gum arabic solution should be ample. Gelatin is a protein; gum arabic is a carbohydrate.
For current sources of Gum Arabic, see Resources, below. Gelatin can be purchased in grocery stores, or from most school chemical supply catalogs.

TIME: 1 45-60 minute period

MATERIALS: Not absolutely necessary, but a sample student worksheet is provided at end of lesson.

TEACHING STRATEGY
IMPORTANT NOTICE: Of prime importance in any introduction or lesson dealing with the possible scenarios of a natural origin of life (as opposed to a supernatural cause) is to make clear the distinction between evolution and the origin of life. These are not necessarily parts of the same process. Mechanisms operating when life came into existence may be quite different than the mechanisms which cause and control evolution. In addition, every indication is that life began billions of years ago, left little if any evidence of the process (no "molecular fossils"), except living organisms, and has not taken place since. In contrast, evolution (which provides an explanation for the diversity of life) has left fossil indicators of past lives, and shows every indication of going on today and into the future. Therefore, evolution is much easier to study, with an abundance of various types of evidence. With the continuation of research going on today, we may indeed eventually have enough circumstantial evidence to describe a plausible scenario for how life began. Perhaps someone in your class will be a part of that discovery....if they are curious, and want to know. Encourage that!

[NB: The concept of "special creation" combines these two processes: life in all its forms was created by divine power all at once. So if we can show convincingly that evolution has produced the diversity of life, we are left with only the question about how life began. Some people believe that God did that, and perhaps

set in motion the process we call 'evolution' as the means for creating the diversity of life. And that's ok to believe. But since, for reasons discussed elsewhere, in our study of the nature of science, we cannot use supernatural forces to explain natural phenomena; we must try to find natural explanations, which can be tested and potentially be disprovable. So scientists ARE looking for clues and exploring various plausible scenarios which would explain how life MIGHT have begun in some natural way.]

As a matter of fact, here is a very logical transition from an early solid evolution introduction to a separate consideration of the origin of life. Upon finishing the evolution intro unit, raise the point: "If evolution shows us how life has changed over eons of time, how different species began, the next question is 'how did life begin?'. We're going to take a look at some of the ideas scientists have explored, and some of their evidence. But first, we need to get familiar with some basics of the chemistry of life, of some molecular biology!."

1. This lesson can be used after studying major organic groups (proteins, carbohydrates, lipids, nucleic acids) and the pH concept, and/or as an introduction to ideas and studies bearing on the origin of life. It's also very helpful if students have done some microscope work, and especially have had a chance to see some live amoebas.
2. You can announce that "we are going to create life! (well, almost)". At least, we will take a look at how easy it is to produce some of the structural and behavioral elements of a living cell, using very simple materials, something analogous to the likely conditions on the early pre-life earth.
3. You might want to precede the lab with some discussion of the Miller experiment (simulating one pre-life scenario of simple gases exposed to electrical discharges, producing amino acids and other organic compounds, and Fox's similar work with amino acids forming proteinoids which, when mixed with water, produced tiny coccus-like microspheres, some even looking like dividing cells. See the list of resources for more recent studies.
4. Prepare the mixes and lab set-ups the day before the lab. You might want to assign reading material dealing with origin of life ideas and studies (in your text, taken from another source, or something you have compiled from different sources. It's also helpful for students to read over the purposes, prep and procedure for the lab the night before doing it.
5. Students can work in pairs, sharing much of the equipment. If you have one microscope for each student, be sure everyone prepares his/her own tube, and tries to find coacervates. Not all coacervates produced will have the desired "amoeba-like" appearance, so as you circulate during the lab, exclaim over any preps which happen to look good, and encourage others to take a peek.
6. It is helpful to do a quick demo of the procedure to entire class just before students do the lab.
7. If possible, have a video camera - microscope set up to display (and videotape) the better-looking coacervates found. When such tapes are played back, fast-forward can more clearly reveal the changes in shape, size, and movements (which are usually fairly slow).
8. If you like, you may want to point out to the class that cell membranes today are essentially double layers of phospholipids (fat), with proteins inserted here and there serving various functions which control transport of materials through the membrane, and/or provide identity markers for the cells. Consequently, the carbohydrate + protein ---> coacervate model is not the most accurate portrayal of a likely pre-life scenario. An activity which does show a more plausible scenario is described in the "Extensions" section below. It uses lecithin to produce little vacuoles. You may want to try this, and if it works, have your students try it.

PROCEDURES

1. FOR SAFETY, be sure to wear goggles and aprons. You will be working with dilute acid in dropping bottles, and accidental splattering can occur. BE CAUTIOUS!
2. Set up your microscope and clean a slide and coverslip.
3. Half fill your culture tube with some of the mix, a 5:3 ratio of protein (gelatin) : carbohydrate (gum-arabic). Using your dropper, put a drop of the mix on pH paper. Read and record the pH of the mix.
4. Add a drop of acid to the tube; cover end with stopper (or finger tip) and invert once to mix. It should turn cloudy. If the cloudiness disappears, add one more little drop of acid and invert-mix. Do this until it

remains cloudy. (This should take no more than 1-3 drops total; if you are adding more, there's something wrong; start again). When it stays cloudy, put another sample drop on pH paper. Record the pH.

5. Place a drop of the cloudy suspension on a slide, add coverslip, and search under low power (40-100x) for something which looks like spit (for want of a better description!)... clear irregular bodies with rounded shape, often with tiny bubbles inside. Starting with a small diaphragm opening at first might help, as coacervates often have very thin delicate outlines, and may be hard to see if light is too bright, and the small aperture causes these fine lines to appear thicker and darker. Movement will not be noticed immediately; you might see some after viewing for awhile. You can go to higher power (400x) for closer look. Draw a typical coacervate or two. If you have a chance, take a look at some of the coacervates found by other students.
6. Add drops of acid (invert-mixing after each drop), count the drops until extreme cloudiness disappears (2-3 additional drops). Record the pH.
7. Before doing the lab, try predicting the approximate pH with each added drop; record these in the "predict. pH" column of your data table. Also, if your text has a picture of a coacervate, draw it on your worksheet, as a "predicted coacervate"
8. When finished with the lab, clean up and begin the discussion questions.

ASSESSMENT

1. Circulate during lab; observe efforts of all students. Take a look at as many preps (in microscope) as you can.
2. Collect the worksheets (or lab report); check especially for reasonable predictions, and logical answers to discussion questions (based on their background to date).
3. Return checked worksheets, and discuss with class.

EXTENSIONS & VARIATIONS

The SETI Institute (Search for Extraterrestrial Intelligence), with support from the Hewlett-Packard Corporation and Foundation for Microbiology, is currently developing an integrated, technology-based high school science curriculum based on the Origin of Life and Evolution at 5 levels. The project is called "Voyages Through Time", and should be available mostly in CD-ROM format, sometime in the next year or so.

One of the lab activities to be included in the SETI materials involves the formation of membranes from the mixing of water with phospholipids (e.g. lecithin). This simple exercise is a much better simulation (than the coacervate lab) of what an early pre-life mechanism for making cell membranes must have been like, as modern cell membranes ARE double-layers of phospholipids. Two methods for visualizing vesicle formation will be included: fluorescent dye method, and colored water method. A pre-development version of the colored water method is available here (just click, then press "back" to return to this page). Try it out, and do give us (SETI and ENSI) feedback regarding its success and/or problems or questions.

Try the Phospho-Lipid lab suggested at the end of this lesson. Current research suggests that early phospholipids are probably more likely precursors to the earliest cell formations (see Deamer, below).

OTHER RESOURCES

deDuve, Christian. Vital Dust - The Origin and Evolution of Life on Earth. 1995. Basic Books (Harper Collins Publishers, Inc.).An excellent treatment of the current status of the now rapidly growing body of research in this area. $15 at Borders.

Deamer, D.W., and Barchfield, G.L. "Encapsulation of Macromolecules by Lipid Vesicles Under Simulated Prebiotic Conditions." 1982. Journal of Molecular Evolution. v.18, pp.203-206. Springer Verlag, New York

Gum Arabic powder (or Gum Acacia... same stuff): Currently available in 100 gram quantity for $8.60 from Sargent Welch or Wards Natural Science (do Google search for company, then search site for Gum Acacia).

ATTRIBUTIONS

1. Original Source: BSCS Blue: Biological Science - A Molecular Approach. 1996 (5th edition), and all previous editions. Investigation 4C: Coacervates (pages 630-631)
2. Modified by: Larry Flammer (1964)
4. Reviewed / Edited by: Martin Nickels, Craig Nelson, Jean Beard :
5. Edited / Revised for website by L. Flammer 6/30/99

ANOTHER WET LAB ON THE ORIGIN OF LIFE

PHOSPHOLIPID VESICLE FORMATION

From Preliminary Draft of Planned Activity for the SETI Project: Voyages Through Time

Materials:

microscopes, slides, coverslips, Lecithin (from local pharmacy) food coloring water eye dropper paper towels

Directions:

Students can work in teams of 3-5. One drop of lecithin solution is placed on a slide, and one drop of colored water is placed next to the lecithin solution. Coverslip is placed so it covers both the lecithin and water. Students should examine the boundary between the water and lecithin at the start of the class and periodically during the rest of the period, drawing a picture of what they see each time. They should be allowed to switch between low, medium and high power, but should label each sketch made with the magnification power used.

Thirty minutes into the period, students should exchange the colored water for clear by placing a piece of paper towel along the water end of the coverslip to draw out the colored water. A drop of clear water should be added at the lecithin end and be sucked through. Repeat as needed to get fairly clear water around the vesicles that have formed. They should be visible as colored spots containing undiluted water and food coloring. Sketch a few vesicles.