

# Spontaneous Generation vs. Biogenesis

Classic Experiments by Redi, Spallanzani, and Pasteur



## Introduction

Where do living things come from? Do they arise from non-living materials, or can they only come from pre-existing living things? Recreate three classic experiments that helped to disprove the theory of spontaneous generation.

## Concepts

- Biogenesis
- Spontaneous generation
- Sterilization

## Materials (for each demonstration or group)

Bleach solution (sodium hypochlorite), 10%, 400 mL	Marker or wax pencil
Chicken, beef or liver, $\frac{1}{3}$ " $\times$ $\frac{1}{3}$ " cubed, 2	Plastic tubing, $\frac{1}{8}$ " $\times$ 1"
Nutrient broth, powder, 2 g	Plastic tubing, $\frac{1}{8}$ " $\times$ 2"
Water, distilled or deionized	Plastic tubing, $\frac{1}{8}$ " $\times$ 3"
Autoclave or pressure cooker	Plugs, foam, 21–26 mm, 10
Beaker, 500-mL	Stirring rod
Cork borer, $\frac{1}{8}$ "	Tape
Gauze, 1" $\times$ 1"	Test tube rack
Gloves, latex, or other disposable type	Test tubes, 25 $\times$ 150 mm, 9
Graduated cylinder, 25 mL	Tongs
Hot plate	

## Safety Precautions

*Be sure to follow directions carefully when using an autoclave or pressure cooker. Sodium hypochlorite (bleach) causes skin burns and is toxic by ingestion. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Follow all laboratory safety guidelines and wash hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.*

## Procedure

### Part A. Francisco Redi's 1668 experiment

*Hypothesis:* Living matter always arises from pre-existing living matter.

1. Label two test tubes "A" and "B." Place a piece of meat in each test tube.
2. Allow test tube "A" to remain open.
3. Place the gauze over the top of test tube "B." Secure the gauze with a rubber band.
4. Place the two test tubes undisturbed, in a safe place, out of direct sunlight, until maggots or fly eggs are visible on the meat. *Note:* The test tubes may be placed outside in a sheltered area where flies are likely to locate them. The life cycle of a fly is 7–10 days in warm weather.
5. Record the date and initial condition of the meat in the test tubes on the first day. Have students predict what they think will happen over a period of time. Ask them to clarify their hypotheses and possible results as they make their predictions.

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6. Record the date and daily observations of the test tubes. Record all results and have a complete discussion about the origin of the maggots.

### Part B. Lazzaro Spallanzani's 1767 experiment

*Hypothesis:* Germs, then called *infusorial animalcules*, present in air cause fermentation and putrefaction.

1. Prepare 250 mL of nutrient broth solution by dissolving 2 g of powdered nutrient broth in 250 mL of distilled water. Heat the solution slightly to completely dissolve the powder. The broth should be a clear, golden color and not cloudy.
2. Label two test tubes "C" and "D." Pour 20 mL of nutrient broth into each of the test tubes.
3. Place the test tubes into the boiling water bath. Place foam plugs at an angle in the mouth of the test tubes. Wait 15 minutes. *Note:* Spallanzani boiled a larger volume of broth for 45 minutes and fused the glass necks of the vials.
4. After 15 minutes, remove the test tubes from the boiling water bath and insert the foam plug into test tube "C." Remove the foam plug from the top of test tube "D."
5. Place the two test tubes out of direct sunlight, undisturbed, in a safe place with few drafts, until the broth in one of the test tubes becomes cloudy.
6. Record the date and initial condition of the nutrient broth in the test tubes on the first day. Have students predict what they think will happen over a period of time. Ask them to clarify their hypotheses and possible results as they make their predictions.
7. Record the date and daily observations of each test tube. Record all results and have a complete discussion about the origin of the microbes after the broth becomes cloudy.

### Part C. Louis Pasteur's 1864 experiment

*Hypothesis:* Germs are found as solid particles in the air.

1. Use the cork borer or a dissection probe to create a 1/8" hole through three foam plugs.
2. Pour 20 mL of nutrient broth into each of five clean test tubes.
3. Autoclave the nutrient broth-filled test tubes with solid foam plugs inserted in the tubes, as well as the three one-hole foam plugs, in an autoclave or pressure cooker according to the manufacturer's instructions. *Note:* Allow a pressure cooker to cool completely before releasing pressure.
4. Chemically sterilize the three pieces of plastic tubing by soaking them in 10% bleach solution for 20 minutes before use.
5. After sterilization, remove the foam plug from one test tube, leaving it open to the environment.
6. Leave the solid foam plug inserted into one of the test tubes.
7. Put on the pair of disposable gloves. Wet a paper towel with the 10% bleach solution. Wipe the palms and fingers of the gloves with the bleach solution.
8. Insert each piece of plastic tubing into a one-hole foam plug. *Note:* Only handle the portion of the foam plug and tubing that will remain outside the test tube.
9. Replace the solid foam plug in each of the three remaining test tubes with one of the tubing-filled foam plugs.
10. Use tape to form the 2" piece of plastic tubing into a "J" shape. See Figure 1. Use tape to form the 3" piece of plastic tubing into an "S" shape. See Figure 2.
11. Place the flasks out of direct sunlight undisturbed, in a safe place with few drafts, until the broth in one of the test tubes becomes cloudy.
12. Record the date and initial condition of the nutrient broth in the test tubes on the first day. Have students predict what they think will happen over a period of time. Ask them to clarify their hypotheses and possible results as they make their predictions.
13. Record the date and daily observations on each of the test tubes. When the broth becomes cloudy in one of the test tubes fitted with a plastic tubing top, record the results and have a complete discussion about the origin of the microbes.

### Disposal

Please consult your current Flinn Scientific Catalog/Reference Manual for general guidelines and specific procedures governing the disposal of laboratory wastes. All test tubes should be autoclaved or chemically sterilized before disposing of the media contents, Flinn Biological Waste Disposal Method Type 1.

### Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

***Unifying Concepts and Processes: Grades K–12***

Evidence, models, and explanation  
Evolution and equilibrium  
Form and function

***Content Standards: Grades 5–8***

Content Standard A: Science as Inquiry  
Content Standard C: Life Science, reproduction and heredity, regulation and behavior  
Content Standard G: History and Nature of Science, science as a human endeavor, nature of science, history of science

***Content Standards: Grades 9–12***

Content Standard A: Science as Inquiry  
Content Standard C: Life Science, biological evolution, behavior of organisms  
Content Standard G: History and Nature of Science, science as a human endeavor, nature of scientific knowledge, historical perspectives

### Tips

- Redi continued his experiment by placing living or dead flies in sealed jars and noting the outcome.
- Decrease the time for growth in Pasteur's experiment by placing the end of the plastic tubing into the nutrient broth.
- Pasteur also proved that microorganisms cannot travel long distances without the assistance of air current or gravity. Microorganisms fall straight down via gravity or are carried in all directions by air currents.
- Tyndallization can be used if an autoclave is not available. Boil the nutrient broth for 20 minutes, allow the broth to cool, then incubate at 37 °C for a day. Repeat the boiling, cooling, incubation procedure for three consecutive days, followed by boiling again. The three incubations force many of the heat-resistant spores to grow due to heat shock. These newly growing bacteria and fungi are killed in the next boiling step. The procedure only works for the nutrient broth. The test tubes and other apparatus must be chemically sterilized with 10% bleach solution.
- A 10% bleach solution is actually a 0.525 w/v% sodium hypochlorite solution.
- Spontaneous Generation, Flinn Catalog No. FB1437, contains bent glass tubing that can be used instead of the plastic tubing to recreate Pasteur's experiment.

### Discussion

The test tubes in this activity represent the classic experiments of Francesco Redi (1626–1697), Lazzaro Spallanzani (1729–1799), and Louis Pasteur (1822–1895). These three scientists and many others sought to verify that living matter arises only from other living matter. The experiments are historically interesting since spontaneous generation was the accepted theory of life for hundreds of years. *Spontaneous generation*, also called abiogenesis, is the theory that life can originate from non-living matter. Redi's experiment was one of the first controlled, documented, scientific experiments to be conducted. However while Redi conducted his experiment, spontaneous generation remained the popular theory on the origins of new living organisms for an additional two hundred years. Redi's hypothesis, that living matter always arises from pre-existing living matter, came to be known as *biogenesis*. Several scientists tested both biogenesis and abiogenesis over the next two hundred years or so. By the time Spallanzani decided to retest abiogenesis, scientists realized that germs or *infusorial animalcules* existed and could only be seen with the aid of a microscope.

Spallanzani sought to test whether Redi's hypothesis was true for these microscopic germs if a broth was boiled to kill all of the germs and the broth was then sealed so that no new air or germs could reach the broth. Spallanzani designed an experiment in

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which broth was boiled for 45 minutes in a flask that was under a slight vacuum and then fused the top of the flask to seal out both air and germs. Although no microbes grew, other scientists argued that microbes may only spontaneously generate if there is air present in the broth.

Many scientists devised experiments that allowed air to mix with the broth but they typically involved a filter. It was not until Louis Pasteur's experiments, two hundred years after Redi, that scientists concluded living organisms arise only from other living organisms. Pasteur devised an experiment that allowed air to freely interact with the broth while keeping the germs out of the broth. In his most famous experiment, Pasteur created three glass necks for the broth flasks. The straight neck allowed germs to "fall" into the flask of broth. The second flask had a "J" shaped neck that allowed air to enter the flask but the germs were unable to "fall" into the neck of the flask. The neck in the third flask was shaped like a long "S." Germs were able to fall into the neck, but the upward slope trapped them. Pasteur boiled the flasks of broth to kill any germs they initially contained. He stored the flasks in an area that had few drafts. The straight neck flask became cloudy with time. In both the "J" and "S" necked flasks, the broth remained clear. Pasteur tipped the "S" necked flask so the broth contacted the dust that had accumulated in the neck. The broth in this flask became cloudy after a few days. Several bottles of broth originally prepared by Pasteur still exist today without bacterial growth—these include samples at the Pasteur Museum in Paris, France, and the Department of Pathology Museum at University of Sydney, Australia.

## References

<http://aleph0.clarku.edu/huxley/CE8/B-Ab.html> (accessed October 2007)

<http://brunelleschi.imss.fi.it/museum/esim.asp?c=300478> (accessed October 2007)

<http://www.whonamedit.com/doctor.cfm> (accessed October 2007)

<http://www.chemheritage.org/educationalservices/pharm/antibiot/readings/spogen.htm> (accessed October 2007)

**Materials for *Spontaneous Generation vs. Biogenesis* are available from Flinn Scientific, Inc.**

Catalog No.	Description
FB1437	Spontaneous Generation— Origin of Microbes Demonstration Kit
AP8373	Plastic Tubing, 1/8-inch i.d., 10 feet
N0094	Nutrient Broth, 8 g

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.