# Antidote for Formalin

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Under the pressure of many duties and responsibilities, the busy biology teacher is often tempted to order preserved materials from a supply house, rather than to secure and care for living things. The temptation should probably be resisted. In too many courses the laboratory appears as a well-stocked morgue. The perfume of formalin is in the air! It is a sad commentary that such courses, supposedly dealing with "Life Science," are actually dedicated to the dead. To be sure, formalin has its uses, but most teachers well know that living plants and animals in the laboratory provide *an antidote for formalin* and greatly increase student interest in biology.

The main thesis of this paper is that more living material should be used in the biological laboratory. Even when the main work is devoted to dissection, it should be lightened by one or more demonstrations or experiments involving living things. For example, a laboratory period devoted chiefly to the dissection and tracing of the circulatory system in preserved specimens should be highlighted by a demonstration of the circulating blood in a living animal. Of course this procedure is standard practice in a great many courses. Nevertheless, the importance of using living materials can scarcely be over emphasized.

The invertebrates offer abundant opportunity for the use of living material. The following suggestions, with one exception, relate to invertebrate animals. The demonstrations and experiments selected are easy to prepare, require no special apparatus, and are reasonably sure to produce satisfactory results. Some of the methods can be documented. Others have reached the author from forgotten sources, via the "grapevine," doubtless much modified in transit.

## Protozoa

The Protozoa provide an enormous number of opportunities for demonstration and experiment, as is attested by the voluminous literature on the subject. The few suggestions presented below are selected on the basis of availability of material and ease of preparation.

*1. Mixed Cultures.* Small masses of pond scum and organic debris from a ditch, pool, pond, or stream should be placed in finger bowls of water, preferably from the same source. Protozoans, crustaceans, and annelid worms will be noted, usually in small numbers. As decay proceeds, these dishes become teeming cultures, and certain forms become dominant. As conditions change, dominant forms decline and are replaced by others. These cultures may be used for a study of population sequences, and of pH changes as the solutions age. They may be used as stocks for the subculturing of selected species. A medium suitable for subculturing any one of several species may be prepared by boiling four or five wheat kernels in 75 ml. of pond water and allowing the solution to cool. The culture fluid should then be seeded with the desired organism. If a culture is exposed to sunlight it will commonly turn green, as a result of the rapid population increase of algae or any euglenoid forms that may have been present in the seeding fluid. If this is not desired, it is advisable to keep the culture in subdued light.

2. *Cultures of Vorticella.* It has been observed that, under normal pond conditions, such protozoans as *Vorticella, Carchesium,* and *Epistylis* are commonly attached to the body surfaces of tadpoles and other aquatic animals. Cultures may be made by placing crayfish or large tadpoles in finger bowls of pond water. Within a day or two, a thin brown scum appears on the surface of the water, and remains for a few days. Study of this scum often reveals the presence of *Vorticella* or other peritrichs. The quick coiling and slower uncoiling of the contractile stalk of *Vorticella* may be readily seen. From descriptions given by Leeuwenhoek, it has been inferred that this animal was the first protozoan that he saw.

3. *Cultures of Paramecium.* Ordinary hay or wheat infusions are useful for rearing *Paramecium* in moderate numbers. For dense cultures of this protozoan, bean-seed infusions may be used (Cole 1935). Four-inch finger bowls containing from 50 to 100 ml. of water are made ready. To each is added one dry bean which has been cut transversely to expose the interior of the cotyledons. The new cultures should be seeded immediately, using about two ml. of hay infusion. The dishes are then stacked and a cover applied to reduce evaporation. An occasional culture will turn sour and the animals will be destroyed. In the others a thick, ropy, gelatinous, bacterial mass develops, and the number of Paramecia increases enormously. The mass should be broken if it grows completely across the surface of the culture. Along the edges of the bacterial masses, the Paramecia become so dense that they appear as cream colored borders. When a drop from such a culture is placed upon a slide, the animals move sluggishly and may be readily studied under high power. A few drops placed in a depression slide and allowed to concentrate through evaporation give a spectacular picture when projected upon a screen.

4. *Quieting Paramecium.* For prolonged study of the structure and many of the activities of *Paramecium,* it is essential that the animal be kept relatively quiet. Many ways have been suggested. Most anesthetics distort or destroy the animal; mechanical methods of retarding movement are usually more satisfactory. The gelatinous nature of the bean-seed infusion, mentioned previously, is a factor which makes the animals move slowly. A one per cent suspension of methyl cellulose in water gives similar results. A drop of this fluid mixed by use of a needle point with a drop of the culture in no way harms the animals. Minute masses of absorbent cotton or of frayed lens paper provide small areas in which Paramecia will be trapped. In any one of these methods, numerous air bubbles may be introduced to limit even further the movements of *Paramecium.* This is accomplished by holding the cover glass about an inch above the preparation and allowing it to drop into place.

The depth of the preparation is another important factor for success in studying the details of structure and activity in *Paramecium.* A "high" preparation allows too much vertical movement of the animal. A "low" preparation is preferable, in spite of the fact that it may dry out sooner. Drying is reduced by using a syringe to make a ring of petroleum jelly on a slide and placing the culture within the ring (Buck 1943). The coverglass is then applied, and gently pressed into position with the point of a needle; a high, medium or low preparation may thus be secured.

5. *Protoplasmic Movements.* The cyclic movements of the food vacuoles in *Paramecium,* and the streaming movements of protoplasm in *Ameba* may be clearly shown in a low preparation by the use of darkfield methods. The mirror of the microscope is moved to one side so that only lateral light enters the preparation, and the background appears dark. Or, the mirror may be deflected so that no light enters the preparation, and sidelighting secured by use of a spot light. Under such conditions, the granules in the protoplasm appear as bright spots, and their movements may be readily followed.

6. *Ingestion in Paramecium.* An ingenious method for studying ingestion has been devised by Buck (1943). Since the amounts he gives provide a larger volume of reagent than would be used by small classes, his directions for preparation have been somewhat modified here. A piece of fresh yeast the size of a pea is boiled in 10 ml. of water to which a trace of congo red dye has been added. Excess dye should be avoided. The cooked yeast should be pink, not red. A syringe is used to make a ring of petroleum jelly on a slide, and a drop

of *paramecium* culture is placed within the ring. Stained yeast is introduced by needle point into the culture. A coverglass is gently pressed down upon the preparation. In spite of the fact that the yeast plants are much larger than the usual food, the Paramecia ingest them readily, and numerous food vacuoles gorged with yeast are formed. Because of the acid condition of the food vacuoles, their contents gradually turn from pink to purple, and at pH 3 become deep blue. The animals become sluggish, gather near air bubbles and bacterial masses, and may there be studied under high power. Movements of the cilia, operation of the contractile vacuoles, and protoplasmic movements may be easily seen.

Acid fuchsin is another dye useful for the study of the feeding reactions of *Paramecium* (Cole, 1934a). A double drop method is used. The slide is ringed with petroleum jelly, and a drop of culture fluid and a drop of one per cent acid fuchsin are placed side by side within the ring. The coverglass is applied and gently pressed into the petroleum jelly until a low preparation is secured. The pellicle of *Paramecium* is impermeable to the dye, but the dye is absorbed on the pellicle in a thin, perhaps monomolecular, layer which gives the animal a pale luminous green color. The surrounding fluid is pink and bacteria are stained a deep red. Under high power one can observe the pink water being swept down the gullet, the red bacteria caught in the swirling currents in the food vacuole, the enlargement of the food vacuole, and finally, the pinching of the tip of the gullet which frees the food vacuole and permits the formation of a new one.

7. *Rate of Vacuole Formation.* If the pellicle of *Paramecium* is permeable to a dye, the staining effects are quite different from those resulting from the use of an impermeable dye. For example, when neutral red 1:5000 is added to a slide of Paramecia, all food vacuoles are stained—red, orange, or yellow, depending upon their pH. In the case of acid fuchsin, the gullet of *Paramecium* is the only avenue by which the stain can enter the interior of the animal. The dye is only slightly toxic. Consequently, a drop of one per cent stain may be added to a drop of culture on a slide, with no apparent ill effects on the protozoans. If such a preparation is made, covered, and then quickly examined under the microscope, it will be noted that only two or three food vacuoles appear red. These are the ones which were formed during the brief time elapsing between the application of the dye and the examination under the microscope. It is possible to time the formation of food vacuoles, and determine the rate of their production.

8. *Extrusion of Trichocysts.* Trichocysts, when extruded, are much longer and thicker than is generally appreciated. The act of extrusion is a spectacular sight and may be easily observed. The double drop method is used. A drop of 1:2000 methylene blue solution and a drop of rich culture are placed side by side, but not touching, on a slide. When the coverglass is applied, the circle appears half blue and half white. The microscope should be focused on the boundary line. Here and there animals will be observed to enter the blue zone. In favorable cases, the trichocysts of the anterior portion, or even of the whole body, will be extruded and instantly stained dark blue. Sometimes the animal retains enough vitality to withdraw, leaving a heap of darkly stained trichocysts resembling a pile of jackstraws. The process may be retarded in individual cases, and one may observe individual trichocysts "explode," one after another. The analogy between this and the popping of popcorn is suggested.

The extrusion of trichocysts, as seen under the compound microscope, suggests that the phenomenon is analogous to the squeezing of toothpaste from a tube. But studies of the trichoycst under the electron microscope have shown that it is a minute preformed structure (Jakus, Hall, and Schmitt 1942; Jakus 1945; Jakus and Hall 1946). Under suitable stimulus it elongates somewhat after the manner of a jack-in-the-box.

9. *Demonstration of the Macronucleus.* Many dyes, including methylene blue, will stain the nucleus of cells. But methyl green is said to be specific for chromatin. One gram of dry stain is dissolved in 500 ml. of one per cent acetic acid. The macronucleus of *Paramecium* stains bright green. The cytoplasm often appears gray or lilac, presumably due to traces of methyl violet (Conn 1953). Methyl green will also cause extrusion of trichocysts; the effect is instantaneous.

*10. Reactions of Paramecium.* The reactions of *Paramecium* to various stimuli have been extensively studied. The classic work of Jennings (1906) and Mast (1911) laid the foundations for many of these studies. Calkins and Summers (1941) and Wichterman (1953) deal extensively with protozoan structure and behavior.

Many reagents can be introduced under the coverglass of a preparation by use of a pipette with the glass tip drawn out into a very small tube. When 0.5 per cent NaCl is introduced into the middle of a preparation on the slide in this manner, the avoiding reaction of *Paramecium* causes the salt solution to remain free of animals for some time. If 0.02 per cent acetic acid is used, the animals show a positive response and are soon thickly gathered in the acidic area. Thermotropism may be shown by placing two metal blocks, one chilled in the refrigerator, and other warmed to about 30 degrees C., on the microscope stage and placing a slide upon them so that one end is chilled and the other warmed. Several drops of culture are then flooded over the slide. Within a few minutes, the Paramecia will be concentrated in a zone nearest the optimum temperature (26 degrees C.).

The reaction of *Paramecium* to slowly diffusing dyes and other chemicals and to the passage of an electric current may be demonstrated by the use of comparatively simple apparatus. A useful material for making such equipment is methyl methacylate polymer, such as Lucite (Cole 1938). It may be conveniently cut to the size of a microscope slide. Minute pits, made with a hot needle, may be used to hold Paramecia within narrow limits of movement. A long trough gouged or ground out of a piece of this plastic makes it easy to study the free movements of *Paramecium.* Two depressions ground in a piece of plastic and connected by a minute shallow groove provide a device for the study of the effect of diffusing chemicals. One depression is filled with culture, the other with the reagent, and the two connected by laying a thread, to serve as a wick, in the groove between them. By using chloroform as a solvent, edges may be built upon a flat piece of plastic, thus forming a shallow box suitable for studying the response of *Paramecium* to weak electric currents.

## Coelenterates

The suggestions relative to this phylum are restricted to *Hydra,* since the use of marine forms is feasible in a limited area only.

*11. Feeding.* This is a widely used demonstration, and is almost always successful. *Hydra* feeds freely on the crustacean, *Daphnia.* The size of the latter determines to some extent the results of the experiment. Large specimens will often shred *Hydra* by rapid movements of the appendages. Medium-sized crustaceans are readily ingested. Fill the cavity of a depression slide with water, add a single *Hydra* and one or more *Daphnia.* Observe under low power. As soon as the tentacles make contact, the crustacean struggles violently. Sooner or later the prey is drawn toward the mouth and engulfed. The heart beat and movements of the appendages of *Daphnia* continue for some time after the animal has been forced into the gastrovascular cavity. Some time later the indigestible residue is regurgitated.

*12. Regeneration Hydra.* May be cut in many ways to demonstrate regeneration. One of the simpler ways is to cut off one or more tentacles. For this purpose the edge of a round coverglass is rolled over the base of the tentacle. Regeneration requires several days, and during that time *Hydra* should be kept in cool, clear water.

*13. Discharge of Nematocysts.* The actual discharge of nematocysts may be observed by placing *Hydra* on a slide in a drop of water, adding a coverglass, and allowing methylene blue 1:2000 to diffuse until contact with the animal is made.

*14. Structure of Nematocysts.* A specimen of *Hydra* should be placed on a slide, a drop of methylene blue 1:2000 added, and a coverglass applied. Both discharged and undischarged nematocysts will be observed. Undischarged penetrants and solvents often stain so as to show the coiled thread *in situ.* Both types of glutinants, however, usually stain solid blue, showing little of the internal structure.

## Flatworms

Several types of flatworms are usually available, and provide material for interesting demonstrations.

*15. Phototropism in Planaria.* The most widely used flatworm *is Planaria* (*Euplanaria*). *Euplanaria maculata* and *E. agilis* show marked

negative phototropism. The under slide of a petri dish should be covered with paper, one half black and the other half white. About one fourth of an inch of pond water is placed in the dish, and 10 planarians added. The dish should be brightly illuminated by a goose-neck lamp. Fifteen or twenty counts should be made, at one minute intervals, to determine how many animals are on the white surface and how many are on the black area. The two sets of numbers should be totaled. Usually, but not always, the black background is favored. Failure of the animals to show consistent negative phototropism may be due to the fact that the black background absorbs and radiates heat to a greater extent than does the white background. Since planarians seeks cooler water, it may happen that their thermotropic response counteracts or neutralizes their phototropic response. If a glass dish filled with water is placed between the light source and the dish containing the animals, heat will be largely absorbed, and a more consistent negative response to light will occur. Response to light will also be clearly shown if planarians are placed in a dish of cool pond water containing several small flat stones.

*16. Regeneration in Planaria.* Regeneration experiments are feasible if care is taken to keep the dish clean and the water clear and cool. *E. agilis* is excellent for this purpose. The animals are vigorous, the color is black, and regenerated tissue appears white at first, then slowly darkens. This is due to the fact that pigment cells in the old tissue migrate slowly into the newly formed areas. Simple cuts are preferable, such as removal of the posterior third or half of the animal.

*17. Feeding in Planaria.* Feeding experiments with planarians often fail, possibly due to their extreme sensitivity to slight changes in the composition of the fluid surrounding them. It is, therefore, very important to use scrupulously clean slides and pipettes. A specimen should be placed on a depression slide in a few drops of water from the culture. Minute amounts of food, such as cooked egg yolk, cooked liver, or fresh earthworm tissue, should be added. In favorable cases the proboscis is extended, and the passage of food into it may be observed under low power.

*18. Parasitic Flatworms.* Flukes may be found in several places in the frog's body. The lungs are the site of *Pneunoneces ranarum* and related species. Remove the lungs from a freshly killed frog and tease the tissues with needles in 0.6 per cent NaCl. If parasites are present they will be seen clearly. The broad, mottled black and white worms are lung flukes. When mounted on a slide and covered, the movements of the digestive tract and the spawning of eggs may be observed.

Yellowish cysts are commonly present on the stomach, intestine and liver of the frog. These contain immature flukes, which may be freed by teasing.

The urinary bladder of the frog often yields several species of flukes. Several of them are conspicuous because of their large suckers.

Tapeworms are present in many fishes and mammals. Perhaps the surest source is an alley cat, but the complications that may arise from an attempt to secure one may make the venture seem scarcely worthwhile! Rats are also a good source. When the intestine of the freshly killed animal is slit open, the tape worms may be lifted with a bent needle and gentle tension exerted until the scolex tears free from the intestinal wall.

## Roundworms

There is a vast number of species of roundworms, although that fact is often obscured by reliance upon large ascarid worms for demonstration and dissection. Numerous smaller forms are available and may be used to demonstrate the peculiar lashing movements characteristic of this group.

*19. Free-living Roundworms.* Free-living forms may be found in vinegar, decaying fruit and vegetables, rich soil, the bark of trees, and many other places. The vinegar "eel," *Turbatrix aceti* is often present in unsterilized vinegar. Fresh apple juice or fresh vinegar may be inoculated; such a culture will thrive for many months, even in a sealed jar or bottle, provided that a generous air space is left. Decaying apples, and turnips with a "soft" spot usually house a teeming roundworm population. If bark from maples, elms, or oaks is placed in a little water, a good colony of roundworms usually develops. Most free-living forms are relatively small, and little internal detail can be made out, even with the use of dyes. Most dyes, unless dissolved in

alcohol, fail to penetrate the cuticle. Alcohol penetrates the cuticle, but greatly distorts the internal organs.

*20. Parasitic Roundworms.* Parasitic forms are also widespread. Slender *Rhabdias ranae* is usually present in the lungs of frogs. The thicker-bodied *Rhabditis pellio* is present in the nephridia of the earthworm. Under pressure and with reduced lighting, some details of the internal structure may be studied. (For an easy way to remove nephridia from the earthworm, see section 26 of this paper).

## Annelids

Oligochaetes are widespread and easily obtainable from water and soil. The examples given belong to this group. The leech has been omitted, although it does lend itself to a number of interesting demonstrations.

*21. Small Oligochaetes.* Pond cultures commonly yield specimens of *Nais*, *Chaetogaster*, and *Aelosoma.* In all of these worms, the peristaltic movements of the digestive tract can be easily observed. In *Aelosoma* particularly, the attachment of the protractor muscles to the bundles of setae, and the change in position of the setae as these muscles contract can be demonstrated under low power. *Aelosoma* reproduces asexually at a fairly rapid rate. If a single specimen is placed in a depression slide containing culture fluid, and covered with a coverglass, the actual rate of reproduction can be determined. Several new individuals will be formed within a day or two.

*22. Sensitivity of the Earthworm.* The earthworm is much more sensitive to mechanical stimuli at its anterior or posterior ends than it is in the median portions. This may be shown by the use of a needle. The prostomium is especially sensitive to a variety of stimuli—tactile, photic, and chemical. The intact earthworm (*Lumbricus terrestris*) is negative to all except very weak light, to which it is positive. If the ventral nerve cord is cut, the portion of the worm anterior to the incision reacts negatively to light; that portion behind the incision reacts positively (Hess 1924). If the cerebral ganglia are removed, the entire worm reacts positively to light of moderate intensity. Sensitivity to light varies greatly in the various parts of the worm's body. Hess (1925) has shown that the degree of sensitivity varies with the number of photoreceptor cells present in the epidermis. Such cells are abundant in the prostomium, the first four segments, and the caudal segment. The segments near the middle of the body have very few. Photoreceptors are absent in most of the ventral areas.

*23. Locomotion in the Earthworm.* A comparison of the rate of locomotion on wet glass and on wet paper towelling shows the important role played by the setae in the process. The antagonistic action of the circular and longitudinal muscles of the body wall can be demonstrated by cutting a small transverse slit and a small lengthwise slit in the dorsal surface posterior to the clitellum (Welsh and Smith 1946). When the circular muscles contract the lengthwise slit gapes; when the longitudinal muscles contract, the transverse slit gapes.

*24. Effects of Narcotics.* A five per cent aqueous solution of urethane (ethyl carbamate) is useful as a gentle anesthetic. Earthworms placed in this solution become limp and insensitive in two or three minutes. Considerable mucus is secreted, but it does not coagulate. This reagent is excellent for demonstrating the circulation of the blood, and for any operations requiring complete recovery. The animal is restored to full activity by immersion in running water.

Chloroform vapor, on the other hand, causes extreme contraction of the circular muscles. It may be used to demonstrate autotomy. At random over the length of the worm, but more frequently posterior to the clitellum, the circular muscles of a segment may constrict to the point where the body is severed, or autotomized.

For preparing fully relaxed specimens for immediate dissection, or for preservation, a procedure using naphthalene in alcohol is recommended (Cole 1927, 1928).

*25. Vascular Movements.* For demonstrating the movements of the dorsal vessel and the "hearts," the specimen should be anesthetized lightly in urethane, the dorsal wall cut slightly to one side of the median line, and the body pinned out on a wax pad. The tissues should be kept moist with 0.6 per cent NaCl.

*26. Demonstration of Nephrostomes.* The

specimen is anesthetized in urethane. Then two or three drops of 1:2000 methylene blue should be injected into the body cavity at several points, until the worm is distended. After ten minutes the dorsal wall should be slit, the worm pinned out on a wax pad, and the digestive tract carefully removed. The nephrostomes, which have a special affinity for the stain, can be clearly seen *in situ* (Cole 1925, 1934b). Since a nephrostome projects through the anterior septum of the segment in which the bulk of the nephridium lies, it is difficult, in unstained specimens, to avoid tearing the nephrostome away from the rest of the nephridium. But, when the organ is stained by the method just described, there is little difficulty in making a complete dissection. The nephridium should be transferred to a slide and a coverglass applied. Features readily observable include the general form of the organ, the roundworms infesting it (Section 20), and the beating of the cilia covering the nephrostome.

(To be continued in February 1955 issue)

# Postage Stamps and Biology Teaching

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Teachers must of necessity be constantly on the lookout for new and better methods of presenting learning materials to their students. Many unique and original ideas evolve in answer to this constant challenge. Recently, I encountered a method which, I am sure, is not only unique, but also adaptable and effective. This method involved the use of postage stamps as a teaching device in biology classes.

When I first observed stamps being used as a teaching device it was done as an individual project in a high school biology class. The class was studying the different areas of the world, and how the plants and animals in them differed. It soon developed that most of the students were aware only of the usual run of foreign animals, elephants, tigers, polar bears, and the like; thus their thinking in regards to the fauna of any region was distinctly stereotyped. One boy in the class, however, collected foreign stamps as a hobby and soon adapted this avocation to a project which greatly aided the teacher in correcting some ideas of the class, as well as influencing several other students in the class to take up this educational hobby.

The project, as completed, consisted of several ten by twenty inch cards each representing some region of the world. Displayed on these cards were neatly arranged stamps from one or more countries in that particular region. The stamps selected were ones depicting the animals of a given country. Since many countries have issued very attractive series along this line, a wide and representative cross-section was available for each region. The beautiful colors and pictures, combined as they were with samples of the native language, made the display an immediate hit with the students.

In this first project seven regions were represented: Australia, The Latin American Highlands, The Latin American Tropics, The Cold Northlands, The Old World Deserts, India and the Far East, and Africa South of the Sahara. In the lower left hand corner of the card a small world map was reproduced, and the countries represented on that particular chart were colored. After the stamps and pertinent information had been placed on the card the whole thing was covered with a sheet of cellophane. This protective measure made it possible for the students to pick up and examine at close range the individual cards without damaging or losing the stamps.

The novelty of this presentation method caused an appreciable increase in interest over more conventional methods of teaching this subject matter and, apparently as a result, the students seemed to have less difficulty in altering their preconceived notions to fit the facts. Retention, too, proved high, and once again proved the maxim that interest and learning go hand in hand.

Like any project, the benefit was not all on the part of the class. The student presenting these cards found a way in which he, normally a rather bashful student, could contribute to the class. This, and similar projects which followed on other phases of the course, seemed to develop in him a feeling of self-confidence and I am sure he gained much