maize pollen, grains treated with an alcoholic solution of IKI reveal wide differences in starch content. It may well be that ecological conditions prior to pollen shedding influence the sugar content of the grains. It is not known whether a reduced osmotic value in the grain is due to the use of some of the sugars in increased respiration or whether sugars may be readily changed to insoluble forms and thus play no part in the osmotic force of the cell. More consistent results

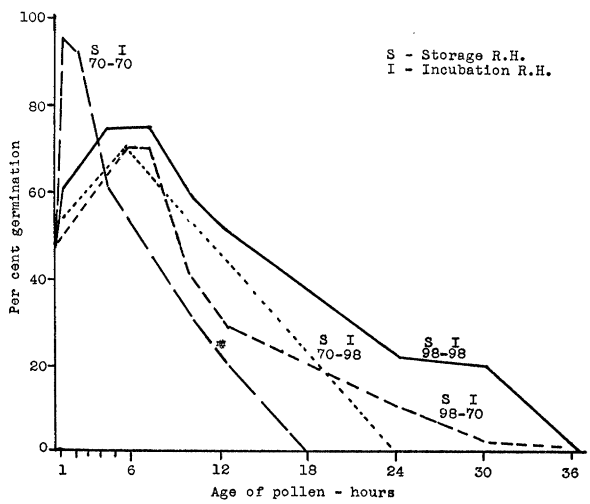

FIG. 1. Effect of age and relative humidity during storage and incubation on the germination of maize pollen.

have been obtained when pollen was taken from cut tassels stored overnight to several days at constant humidity and temperature, perhaps because more uniform osmotic values are obtained with such preliminary treatment.

A major factor in the success of the method is the degree of imbedding of the pollen grains in the still soft agar, and the agar percentage, temperature and cooling rate are important in this connection. Best results have been obtained when the grains were two thirds imbedded and one third exposed to the air. Presumably these conditions favor absorption of both water and oxygen. Covering the pollen has prevented germination. Germinating at 90 per cent. rather than higher humidity reduces bursting due to the formation of free moisture films on the agar. Good germination has been obtained in many experiments at 60 per cent. humidity, although shorter tubes were produced in the drying agar. The data of Fig. 1 show that germination under artificial conditions improved with storage for about six hours after shedding, then declined rapidly. One lot of pollen remained viable after 10 days storage at 8 degrees C. Longer storage life was obtained at the higher humidities, but many lots of pollen stored in nearly saturated air suddenly appeared to become moist and to clump together and thereafter showed no germination.

Maize pollen has germinated poorly on fructose and progressively better on glycerine, glucose and sucrose used at equivalent molarities. Tubes have been produced at pH 4.0 to 8.4. Under optimum conditions the elongation of the pollen tube is sufficiently rapid to be plainly visible under the microscope, and when projected on a screen by a micro-projector so that the grain appeared as large as a grapefruit, the image of the growing point has progressed as much as 1 cm a minute. Tube lengths have frequently reached fifty times the diameter of the pollen grain, although no attempt to stimulate tube growth, as distinct from germination, has been made.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DAILY REMOVAL OF FORMALIN FROM PRESERVED BIOLOGICAL SPECIMENS USED IN CLASS WORK

INSTRUCTORS and students who are exposed repeatedly to the formalin contained in preserved biological specimens often find their laboratory periods extremely disagreeable and, occasionally, hazardous. Induction or aggravation of the common cold, severe dermatitis, bronchitis and asthma are the chief hazards of exposure to formalin. A method is needed to remove this common laboratory preservative.[1]

Dr. Foust, *et al.*,[2] reported the value of a 5 per cent. urea and 1 per cent. ammonium phosphate solution in removing formalin from preserved specimens. Following the directions given in this article, the writers attempted to deformalinize bullfrogs, dogfish, starfish, etc., but failed to obtain satisfactory results. Therefore, we sought to develop another method to achieve the desired result.

After making a series of small-scale tests with some forty laboratory reagents known to react with formalin, we selected sodium bisulfite, $NaHSO_3$, as most closely approximating our objective, *viz.*, of finding a convenient, quick, cheap and efficient method for removing formalin. An aqueous solution of this reagent was entirely suitable in so far as it completely destroyed the formalin odor of preserved specimens

[1] P. H. Pope, SCIENCE, 73: 495, 1931.
[2] H. F. Foust, T. S. Leith, H. M. Tabbut and L. Bowstead, SCIENCE, 83: 498, 1935.

immersed in it within 3 to 5 minutes. The largest specimens required the greatest length of time.

However, because of the acidity of the formalin resulting from the presence of formic acid, sulfur dioxide gas, SO_2, was liberated from the $NaHSO_3$ solution in such quantities as to nullify its usefulness in a classroom procedure. It appeared that this source of SO_2 could be eliminated simply by reducing the H ion concentration of the reactant solution. This was accomplished by buffering the solution with Na_2SO_3.

We may now detail the essential points in the preparation and use of the sulfite-bisulfite solution:

(1) The deformalinizing solution contains 5.7 per cent. (by weight) of $NaHSO_3$ and 3.8 per cent. (by weight) of Na_2SO_3 dissolved in tap water. A deviation of 1 to 5 per cent. from the above figures would probably introduce no serious failure of the solution to function properly. It is our experience that 20–30 liters of the solution will last a full semester in the daily removal of formalin from any specimens in use in a zoology class of 35 students. To prepare 20 liters of solution dissolve 1260 grams of $NaHSO_3$ and 840 grams of Na_2SO_3 in tap water.

(2) Specimens removed from their formalin bath are given a brief preliminary rinsing under the tap, and then immersed in the sulfite-bisulfite solution from 3 to 5 minutes. As many specimens as can be conveniently handled may be deformalinized simultaneously. Following a final quick rinse, the specimens are free of formalin odor and ready for dissection. Large specimens or those which may have been injected with various formalin mixtures may require subsequent short immersions as dissection proceeds.

(3) Failure of the solution after repeated usage to remove the formalin promptly may require the addition of more $NaHSO_3$ just short of the point where SO_2 gas is evolved. Evidence of SO_2 arising during the routine employment of the solution calls for the addition of small amounts of the Na_2SO_3. There is a considerable variation in the actual amounts of $NaHSO_3$ and of Na_2SO_3 in the technical grade of these chemicals. This should be kept in mind and the amount of one or the other reagent increased as may be necessary to give a satisfactory solution. A pH determination of the solution gives a reasonably easy method of ascertaining if it has been properly prepared. The solution of the concentration specified has a pH of about 6.4. One containing insufficient Na_2SO_3 and which may therefore evolve sulfur dioxide, will have a lower pH. One containing an excess of Na_2SO_3 will have a higher pH.

(4) Although certain specimens, frogs for example, may be stored for several weeks in the reactant solution without impairing their dissecting qualities, others such as the dogfish become soft after 5 to 6 days and unsuitable for dissection. In other words, the solution is not a substitute for formalin. After the removal of the formalin, the specimen may be kept in any other satisfactory preservative, or returned to formalin.

(5) The solution should be kept in common glazed earthenware laboratory crocks, as it will slowly attack metal containers.

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A SIMPLE METHOD FOR REMOVING THE PLUNGERS OF "FROZEN" GLASS SYRINGES

THE method of removing the plungers of "frozen" glass syringes suggested by Goff in SCIENCE, Vol. 93, page 602, was of much interest to us. While we make no claim whatever to originality, we feel justified in calling attention to the method of removing the plungers of "frozen" glass syringes used in our laboratory, because of its simplicity and usefulness, and because many persons are unfamiliar with it.

All that is required is a syringe with a plunger of lesser diameter than the plunger of the "frozen" syringe, and equipped with a short hypodermic needle. We often use a 1 cc Yale tuberculin syringe. The needle passes through a small bit of rubber, such as a piece of a wide rubber band which acts as a gasket. The tuberculin syringe is filled with water, and the needle inserted into the outlet of the "frozen" syringe, the piece of rubber making an airtight seal. Water is then forced from the tuberculin syringe into the "frozen" syringe, until the plunger of the latter is free. It may be necessary to fill the tuberculin syringe with water a number of times, but the method almost never fails.

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