perature in amber bottles, no visible change was noted in the crystalline oxalate, whereas pronounced darkening occurred in the monohydrochloride salt. Aqueous solutions of the former could also be maintained for longer periods with less discoloration and precipitation. Furthermore, after standing 48 hours under the above conditions, less precipitation was noted in the aqueous solutions of the oxalate salt than in those prepared from the monohydrochloride salt. Comparative readings with a Klett-Summerson colorimeter showed that the solution of the oxalate salt needed to stand for 72 hours in order to give a reading equivalent to the 24-hour reading of the monohydrochloride solution, thus indicating that the rate of decomposition of the oxalate salt was approximately one-third that of the hydrochloride salt.

The toxicity of 1 per cent solutions of the two salts for the gonococcus was similar. Seventy-five per cent of the strains tested were viable after 5 minutes in either solution.

A slight disadvantage of the oxalate salt, however, was the relatively slower rate at which it went into solution in water at room temperature. This could be hastened by gentle heating.

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Histochemical Method for the Detection of Phosphorylase in Plant Tissues[1]

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Since its discovery in 1939 (*1*), phosphorylase has been found to occur in a number of plant species (*2*). It catalyzes the reversible reaction, starch + phosphate $\rightleftharpoons$ glucose-1-phosphate (Cori ester). The importance of this enzyme in plant physiology is obvious. It represents the first synthesis, *in vitro*, of starch, the most important product of the plant kingdom. A histochemical method for its detection is highly desirable, since knowledge about its distribution and localization would be very helpful in studying the physiology of starch formation in plants.

The success of a histochemical method depends primarily on the formation of an insoluble, stainable reaction product specific to the enzyme. Obviously, neither glucose-1-phosphate nor the phosphate ion can be used in the present case because of their diffusibility, the difficulty of their detection, and complications due to other enzymatic reactions (e.g. phosphatase). Starch, however, answers all the requirements. The question resolves itself, therefore, into finding a plant tissue which is free, or can be made free, from starch.

In an investigation in one of our laboratories[2] it has been observed that soybean contains no detectable amount of starch until one to two days after germination. A histochemical method for phosphorylase can, therefore, be easily devised with this material.

Soybean (*Glycine Max Merr.*) soaked for 12 hours in water was cut into free-hand sections 10–20 μ thick. The sections were incubated at 25°C. in a medium consisting of 1 per cent glucose-1-phosphate in a buffer saturated with toluol. Acetate buffer at pH 6 was generally used. Sections were taken out at frequent intervals and stained with iodine in potassium iodide.

Starch grains can be observed in some parts of the soybean section after 30–60 minutes of incubation, indicating the presence of phosphorylase.[3] That the formation of starch is due to phosphorylase and not to other factors (e.g. phosphatase and amylase) is proved by the facts that (1) no starch can be found until more than 12 hours of incubation if glucose instead of glucose-1-phosphate is used in the medium; (2) no effect is produced by the presence in the medium of M/200 sodium fluoride, which completely inhibits the activity of phosphatase (*3*); and (3) boiled sections give no reaction.

Using the time of appearance of starch and its abundance as a measure of phosphorylase activity, it has been found that, in the soaked soybean, the most intense reaction is located in the rootcap,[4] a less intense reaction is observed in the root tip and the lateral buds, still less in the young leaves, stem tip, and hypocotyl, and the least in the cotyledons.

Starch formation in intact germinating soybean has also been studied, and its relation to the distribution of phosphorylase will be given later in a more detailed report.

The present method is by no means limited to starch-free plant tissues such as soybeans. Most plant tissues can be deprived of starch by keeping them in darkness for a certain length of time. Geranium (*Pelargonium zonale Ait.*) leaves starved for two days in the dark, for example, show a strong phosphorylase reaction in the mesophyll cells after incubation with glucose-1-phosphate.

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3

Use of Aerosol OT in Dissecting Salivary Glands of Mosquitoes Infected With Malaria

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When mosquitoes are dissected in an aqueous medium such as physiological saline, extreme annoyance and difficulty are often experienced because of the tendency of the insects to float on top of the solution and the dissected parts to adhere to the dissecting needles. Since immersing the mosquitoes in ethyl alcohol does not always overcome this difficulty, another surface-tension reductant was sought which would insure thorough wetting of the mosquito and yet not damage the sporozoites which might be present in the salivary glands.

A method frequently used for dissections in this laboratory

[1] This work was started in Kunming, the wartime University site, and was continued in Peiping.

[2] Unpublished results of the Physiology Laboratory, Agriculture Institute, National Tsinghus University, Kunming.

[3] A statement by J. B. Sumner (*Cornell Extension Bull.* 668, 1945) that "soybeans lack phosphorylase" (p. 17), refers perhaps to dormant beans.

[4] Phosphatase activity is strongest in rootcap of grains, according to Glick and Fischer (*Arch. Biochem.*, 1946, **11**, 65–79).

consists of anesthetizing the mosquitoes, removing them to a Syracuse watch glass, placing a drop of 70 per cent ethyl alcohol on each insect, filling the watch glass with distilled water, and finally transferring each mosquito to a drop of physiological saline on a microscope slide for dissection.

The method followed in testing new agents was to immerse the anesthetized mosquitoes in the test solutions and then transfer them, in a drop of the same material, to a slide for dissection.

Preliminary trials were made, employing in turn (1) isoamyl alcohol, (2) capryl alcohol, (3) ethyl acetate, and (4) Aerosol OT (dioctyl sodium sulfosuccinate). The first three compounds were discarded because, in the concentrations used, their immiscibility with water made detection of sporozoites difficult. Aerosol OT, however, seemed to possess the desired properties and was, therefore, examined further.

A 1:100 stock solution of Aerosol OT was made by adding 100 ml. of distilled water to 1 gram of Aerosol OT and allowing it to stand overnight at room temperature. Working solutions were made from this stock by subsequent dilution with physiological saline (0.85 per cent NaCl solution).

As a result of a series of observations using both *Aëdes aegypti* infected with *Plasmodium gallinaceum* and *Anopheles quadrimaculatus* infected with *P. vivax*, a 1:60 dilution with physiological saline of the 1:100 aqueous stock Aerosol OT solution (final dilution of Aerosol OT, 1:6,000) was ultimately found to be optimal.

In order to determine whether the 1:6,000 Aerosol OT solution has a deleterious effect on the sporozoites, the viability of sporozoites from mosquitoes wet and dissected in this solution was compared with that of sporozoites from mosquitoes wet in 70 per cent ethyl alcohol and dissected in saline. *A. aegypti* infected with *P. gallinaceum* were used. Twenty chicks, about one week old, were divided into 4 groups of 5 each. Each chick was injected subcutaneously with the infected salivary glands from one mosquito. The first group of 5 chicks (A) received the infected glands immediately after the glands were dissected in Aerosol OT. The second group (B) was inoculated with infected glands which had been dissected in Aerosol OT and allowed to remain in that medium at room temperature for 15 minutes. Two control groups (C and D) were inoculated with infected glands dissected in physiological saline. Chicks in group C received the glands immediately after dissection, while those in group D were inoculated with the glands which had remained in saline at room temperature for 15 minutes.

All of the chicks in group A showed infection on the 11th day; of those in group B, three showed infection on the 11th day, one on the 13th, and one on the 18th. All of the chicks in the control groups, C and D, with the exception of one which died early in the experiment, exhibited parasites on the 11th day.

In a similar experiment using a 1:4,000 dilution of Aerosol OT, the infections in the groups dissected in Aerosol OT were delayed, only three chicks in groups A and B exhibiting parasitemia by the 36th day. All of the chicks in the control groups showed infection by the 9th day.

These experiments establish the fact that a 1:6,000 dilution of Aerosol OT is a good wetting agent for mosquitoes; the insects can be dissected easily, and if sporozoites are present in the salivary glands, they are readily detected. However, it appears to have a deleterious effect on sporozoites of *P. gallinaceum*, and should be used with caution when the sporozoites of this or other species are to be used for producing infections.

A Quick Method for Filling Curved Glass Apparatus With Liquids

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The usual technique of filling certain types of glass apparatus with a liquid requires the tedious and time-consuming procedure of immersing the apparatus first in hot water and then in cold, the apparatus being connected to the liquid when it is placed in the cold water. This procedure must be repeated many times, and much difficulty is usually experienced in disposing of the last air bubble.

A simple and quick way to accomplish the task is to thread two small, flexible plastic tubes through the curved glass

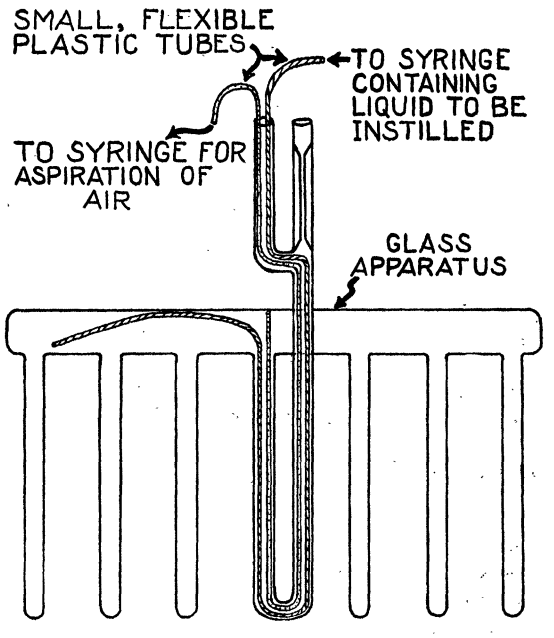

FIG. 1

tubing to the top of the apparatus (the plastic being nonsoluble in the liquid used). The tubing can be passed around bends more easily by repeated short thrusts associated with a twisting motion than by steady pressure. Each plastic tube is then connected to a needle of appropriate size, attached to a large syringe. One syringe is used for instillation of the liquid, and another for the concomitant aspiration of air. After filling the glass apparatus, the plastic tubes are carefully removed.

Using polyethylene tubing furnished by Suprenant Electrical Insulation Company, a glass apparatus designed for use as a thermoregulator was completely filled with toluene in a period of 10 minutes (Fig. 1).

The described technique is of especial value when the layering of solutions is necessary or volatile liquids are being used.