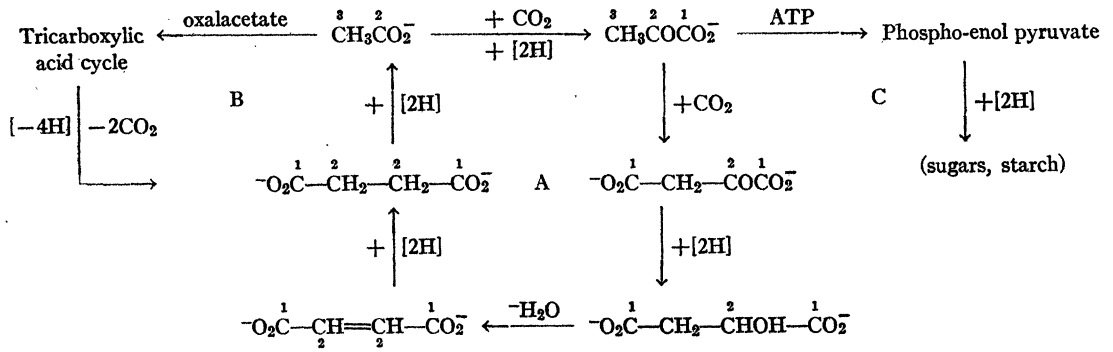
results as well as the observed distribution of radiocarbon in sugar produced by a short photosynthesis (*1*).



Starting with either acetate or pyruvate, the numbers over each carbon atom indicate which carbon atoms are labeled each time around cycle A. The reducing power (indicated as [2H]) is, of course, ultimately derived from the light reaction, and some of it might well be reduced coenzymes I or II. The high-energy phosphate required in these reductions is not explicitly shown in the chart. All or part of it could easily be derived from the combustion of part of the acetate through cycle B.

It should be mentioned that this scheme cannot be a simple reversal of the respiratory system of reactions, since CO_2 derived from respiration of barley leaves (*2*) containing freshly photosynthesized radioactive sugars has a lower specific activity (per mg. C) than the sugar itself. If respiration involves some of the same intermediates as those shown in the chart, the respiratory system must be physically separated from the photosynthetic system.

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IN THE LABORATORY

The Oxalate Salt of P-Aminodimethylaniline, an Improved Reagent for the Oxidase Test

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The cultural method for the diagnosis of gonococcal infection, now a standard procedure (*1*) in most public health laboratories, utilizes the so-called "oxidase test" for rapidly distinguishing colonies of the *Neisseria* from non-oxidase-producing colonies of other genera. The oxidase reaction is considered to depend upon the reaction of an oxidative enzyme with an aromatic amine to produce a series of readily discernible color changes ranging from pink to black.

The dye component heretofore recommended for the oxidase test is the monohydrochloride salt of p-aminodimethylaniline. On standing, this agent deteriorates and becomes discolored, thereby reducing its solubility and the clarity of its aqueous solutions. The precipitate which forms interferes with the separation of oxidase-positive from oxidase-negative colonies in mixed cultures.

Because the oxalic acid salts of aromatic amines are, in general, more stable than the corresponding hydrochloride acid salts, the oxalate salt of p-aminodimethylaniline, $[H_2NC_6H_4N(CH_3)_2]_2 \cdot (—COOH)_2$,[1] was tested for its suitability in the oxidase reaction.

Observations were made on the rapidity with which the dry, crystalline oxalate salt deteriorated at temperatures ranging from 18° to 23° C. One per cent aqueous solutions of the monohydrochloride and of the oxalate salt were compared in the oxidase reaction on chocolate agar plates inoculated with cultures of *N. gonorrhoeae*, with mixed cultures of *N. gonorrhoeae*, streptococci, and diphtheroids, and with cervical and urethral exudates for evidence of gonococcal infection.

A comparative study of the stability of 1 per cent aqueous solutions of each compound was made at temperatures of from 18° to 23° C. Measurements of the oxidative changes in the two dye salts were made at 24, 48, and 72 hours after preparation of the solutions, using a Klett-Summerson colorimeter.

The toxicity of 1 per cent aqueous solutions of the two salts was tested on recently isolated strains of the gonococcus.

The dry, crystalline oxalate salt was more stable than the monohydrochloride salt. After 6 months storage at room tem-

[1] P-aminodimethylaniline oxalate was prepared and supplied by the Research Laboratories of the Eastman Kodak Company, Rochester, New York.

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).