a new approach to the important problem of determining the relation between quantities of chloroplast pigments and plant growth.

In making use of the method for studies concerning the nutritive value and chlorophyll content of plant tissue, the second author of this note found it desirable to modify the color standard by changing the relative proportions of the two dyes. The modified color standard is more satisfactory for general work than the one described previously, since it produces a more perfect match in color with chlorophyll extracts of various plants. It is prepared as follows.

Nine tenths cc of a ½ per cent. aqueous solution of Malachite Green and 11.2 cc of a ½ per cent. aqueous solution of Naphthol Yellow are made up to 5,500 cc with distilled water. The concentration of color in this standard is the equivalent of that produced by 11.873 milligrams of chlorophyll saponified to chlorophyllins and diluted with water to make one liter.

Several investigators have called attention (in personal correspondence) to the variability in different lots of Malachite Green and Naphthol Yellow which may be purchased. Because of this situation, lots of dyes from a number of sources have been compared with the original dyes[3] used in preparing the standard. The following lots of Malachite Green were apparently identical with the original lot: (1) *Malachite Green Crystals,* 1264, Research Laboratory, Eastman Kodak Company; (2) *Malachite Green Crystals,* the Coleman and Bell Company; (3) *Malachite Green,* for histological use, Schultz No. 495, C. I. 657, National Aniline and Chemical Company, "Found satisfactory by commission on standardization of biological stains for above-mentioned purposes."

Malachite Green 0926, Dr. Grübler and Company, Leipzig, Germany, was identical in color but more concentrated than the original lot of dye. *Malachite Green Hydrochloride,* the Coleman and Bell Company, proved unsuitable for this purpose.

The several lots of Naphthol Yellow tested were identical in tint with the standard lot of this dye, but all required filtering except the Martiusgelb of Grübler. One half per cent. aqueous solutions of each lot of dye tested were made with distilled water and allowed to stand for several hours until maximum solution at room temperature was obtained, and then filtered with No. 42 Whatman filter-paper. With this treatment, the following lots of dye gave filtrates identical in color and concentration with the standard lot of Naphthol Yellow: (1) *Naphthol Yellow S,* National Medicinal Products, National Aniline and Chemical Company; (2) the same label as No. 1 but distributed by the Eastman Kodak Company; (3) *Naphthol Yellow,* E & A Biological Stains, B 119, Eimer and Amend.

The *Martiusgelb* of the Dr. Grübler and Company proved less concentrated than the original dye, likewise the *Martius Yellow* (Naphthol Yellow) Schultz No. 6, C. I. 9 of the National Aniline and Chemical Company. The latter dye bears the additional statement, "Found satisfactory by commission on standardization of biological stains, for use in histology, especially the Pianese triple stain." The *Naphthol Yellow* sold by the Fisher Scientific Company was more concentrated than the original standard dye.

It is suggested that workers expecting to make use of the chlorophyll color standard described in the foregoing in any extensive investigations purchase a considerable quantity of the satisfactory lots of dye.

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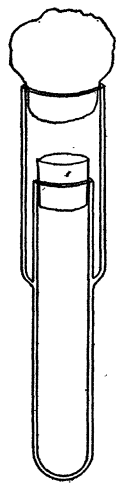
A STORAGE TUBE FOR PLASMA AND EMBRYO JUICE

IN cultivating mammalian tissues *in vitro* the problem of containers for the storage of embryo juice and plasma is often a vexing one since it is difficult to insure effective cooling and sterility and at the same time to facilitate the handling of these substances. In our laboratory we have devised a double-necked tube which has proved very efficient and which may be of interest to others engaged in similar problems, as it avoids these difficulties. The single tube in common use is very subject to infection in the ice pack, and the constant flaming besides being a nuisance hastens clotting. The double tube described by Strangeways, while insuring sterility, is difficult to cool and in operation has the same objections as the single tube. To overcome these difficulties we prepared a tube with a double neck fashioned in such a manner that the outer neck was somewhat longer than the inner one in order that each could be plugged independently. The lower end of the tube being of a single thickness is in direct contact with the ice and insures cooling. The opening of the outer neck is plugged with cotton which may be removed by the fingers. The inner neck-opening is plugged with a sterile cork and can be removed with sterile forceps. This double protection has many advantages that facilitate operation.

Used in place of the usual tube in centrifuging the rat plasma, the double plug insures sterility without flaming and removes the risk of raising the temperature and hastening clotting.

In the operation of mounting the cultures the containers for the embryo juice and the plasma must be opened many times. This operation necessitates flam-

ing each time to avoid infection and is time consuming. Used in this operation the double-necked tube



is supported in the ice pack at an angle of 45°. When the inner plug has once been removed it is left out, the outer cotton plug alone being used. This may freely be removed and replaced many times without flaming. The tube can thus be used throughout the operation (in our case thirty cultures) without removal from the ice pack, with much saving of time and no danger of infection. Even in experiments where heparin is used to prevent clotting and the ice pack dispensed with the double-necked tube saves much time and is an additional precaution against contamination.

As will be seen from the diagram this tube consists of a small test-tube 7½ cms in length, 1½ cms in diameter and rounded off at one end. A collar is fused to this tube about 2 cms from the open end. It projects 3 cms beyond the open end of the inner tube. The jacket has a total length of 5 cms and a diameter of 2 cms.

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SPECIAL ARTICLES

VITAMIN B_2

WHEREAS considerable progress has been made on the concentration of vitamin B_1, comparatively little work has been reported on the purification and concentration of vitamin B_2. Within the last year only two communications, one by Chick and Roscoe,[1] and the other by Narayanan and Drummond,[2] have appeared on this subject. In the first paper a partial separation of the B_2 fraction from B_1 is reported. The process leading to the separation was very complicated, involving many steps. In the second paper, which has appeared very recently, vitamin B_2 is active in daily doses of 0.006 gm.

Over a year ago, we succeeded in separating the B_2 fraction from B_1 in a very simple way. Itself added to a vitamin B-free diet, it did not maintain growth of white rats; with 0.00015 to 0.0002 gm of our vitamin B_1 fraction, it maintained normal growth.

Fraction B_1 is adsorbed on silica gel at pH 3. The filtrate is rich in vitamin B_2, but still contains some vitamin B_1. By precipitation with acetone a material is obtained of which daily doses of 0.015 gm in addition to 0.0002 gm of B_1, both added to the standard diet, suffice to maintain normal growth of white rats. By repeating the extraction six times, a material is obtained from the filtrate of which daily doses of 0.005 gm are required. Finally, when this material is dissolved in water and precipitated with alcohol containing one per cent. of hydroiodic acid, a material is obtained of which daily doses of 0.0007 gm suffice to maintain normal growth of white rats.

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1. Chick, H., and Roscoe, M. H., *Biochem. J.*, 23: 504 (1929).

2. Narayanan, B. T., and Drummond, J. C., *Biochem. J.*, 24: 19 (1930).

THE BEHAVIOR OF WINTER WHEAT IN ARTIFICIAL ENVIRONMENTS

IN physiological investigations of diseased as well as healthy winter wheat plants, frequently it has not been possible to obtain satisfactory results with the plants produced in greenhouses and experimental culture chambers. Such plants produce abnormally long leaves and leaf sheaths, and tillering tends to be reduced during the early growth stage (predormant stage) previous to the stage of winter dormancy as compared with plants grown in the open. These abnormalities have made it practically impossible to study the rosette phase of the wheat-mosaic disease under controlled conditions throughout the year, and to obtain satisfactory heading in winter varieties.

In 1925, studies were started with the definite aim of determining methods for obtaining normal winter wheat, especially during the predormant growth stage in experimental culture chambers.

As a basis for determining normal development, measurements were made on field-grown Harvest