also partially inactivated when the protein was autoclaved in the absence of sucrose, possibly by a reaction of their free carboxyl groups with the free amino group of lysine to give a linkage resistant to enzymatic digestion. Cystine, methionine, and histidine inactivation was primarily caused by a reaction with sucrose to form an enzyme-resistant linkage. As only small amounts of phenylalanine, threonine, leucine, isoleucine, and valine were inactivated, it appears that the amino acids which are inactivated are those with free amino or carboxyl groups, or with other active groups such as the sulfur of cystine and methionine, or the imidazole of histidine.

Representative free amino acids were added to samples of soybean protein and the protein-sucrose mixture before autoclaving. The results are presented in Table 2. No relation between the behavior of free and protein-bound amino acids is apparent. Except for lysine, cystine, and phenylalanine, destruction accounted for practically all of the inactivation. The important point is that, except for cystine which is very insoluble under the conditions of autoclaving, over 45% of each of the free amino acids was destroyed when autoclaved with a mixture of soybean protein and sucrose.

From the results of this investigation it appears that at least three types of reaction are involved in the inactivation of amino acids by prolonged autoclaving of a sucrose containing food or feed, such as soybean oil meal. Lysine, aspartic, and glutamic acids combine with some constituents of the protein, probably the free carboxyl with the free amino groups, to form enzyme-resistant linkages. The amino acids with free amino groups react with sucrose to destroy the amino acids. Protein-bound methionine, cystine, and histidine with sucrose form linkages resistant to enzymatic hydrolysis *in vitro*.

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Improved Apparatus for Radiobiological Syntheses[1]

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Radioactive natural compounds, invaluable tracers for studying intermediary metabolism, are most conveniently prepared by radiobiological methods. Livingston and Medes (*4*) demonstrated that a detached leaf can efficiently photosynthesize $C^{13}O_2$ into C^{13} carbohydrates. Aronoff, Benson, Hassid, and Calvin (*1*) first reported a preparation of radioactive C^{14} glucose; and Putnam, Hassid, Krotkov, and Barker (*6*) have given detailed instructions for preparation. However, the apparatus designs employed by these pioneer workers warrant considerable improvement. A simplified photosynthesis apparatus and procedure, used to prepare C^{14} glucose for poliomyelitis studies, is described in this paper.

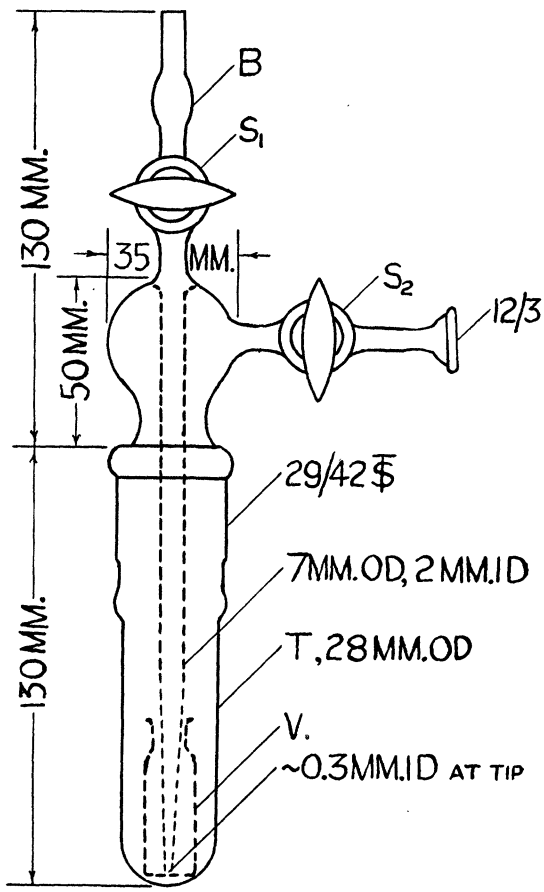

FIG. 1. Photosynthesis vessel.

Tube (T) (Fig. 1) is rinsed with water and left moist. A mature sweet potato leaf (petiole detached, fresh weight about 350 mg), which was removed from a plant in the light about 18 hr earlier, wet thoroughly, wrapped in wax paper and stored in the dark, is now arranged (under side facing in) about the inner surface of the tube. A 6-ml vial (V), containing 50 mg of $BaC^{14}O_3$, moistened to avoid possible scattering, is placed at the bottom of the tube; the standard taper joint lubricated; and the photosynthesis vessel assembled as shown. The vessel is evacuated through stopcock (S_2), 0.5 ml of 3N $HClO_4$ is introduced into bulb (B) by means of a narrow-tipped dropper, and stopcock (S_1) is carefully opened to admit the acid to the carbonate. When the

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acid solution is clear and no longer effervescent, carbon dioxide-free air is admitted through stopcock (S_1) to restore the internal pressure to atmospheric. Finally, the vessel is slipped into a stiff wire holder so adjusted that the vessel is disposed parallel to and 5 cm from a 40-watt fluorescent lamp. The above manipulations can be carried through in 7 min or less.

After photosynthesis has continued for 24 hr, 2 CO_2 absorption vessels, each containing 10 ml of 0.05N $Ba(OH)_2$ solution, 5% in $BaCl_2 \cdot 5H_2O$, are attached as shown in Fig. 2. The second vessel connects with a

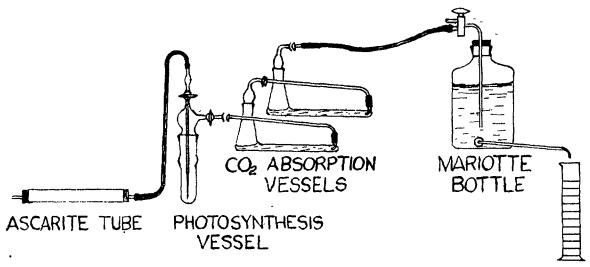

FIG. 2. CO_2 absorption train.

Mariotte bottle, the outlet tube of which is lowered to obtain an air flow of about 10 ml per min through the system when the stopcocks are opened. After 20 min of aeration, excess $Ba(OH)_2$ is back-titrated with standard HCl, using phenolphthalein indicator. Essentially no CO_2 reaches the second absorption vessel.

It was found from seven preliminary runs with inactive CO_2 that an average of 0.27 mg CO_2 was assimilated per mg dry weight of sweet potato leaf in a 24-hr run under the described conditions. For a 10-hr run, 52% of this amount was assimilated; for a 2-hr run, 12%. In every 24-hr run where the amount of CO_2 was not in excess, essentially quantitative (98.8–99.6%) assimilation of available CO_2 took place.

A simplified procedure, based on the fractionation scheme of Hassid, McCready, and Rosenfels (*3*), was used to isolate C^{14} glucose. The leaf was homogenized, filtered, and extracted 1 hr with 5 ml of 80% alcohol. Five ml of 1N H_2SO_4 was added to the extract, which was hydrolyzed for 30 min with simultaneous elimination of the alcohol by distillation. A small excess of hot 10N $Ba(OH)_2$ was added, the suspension slightly re-acidified with H_2SO_4 and brought to final neutrality with solid $BaCO_3$. After centrifuging off the residue and adding 300 mg of carrier glucose, the sugar solution was concentrated under vacuum to a syrup, taken up in 10 ml of 95% alcohol, brought to turbidity with ether, and set aside for glucose crystallization.

The extracted leaf residue was boiled 15 min with 5 ml of acid alcohol (4 ml of concentrated H_2SO_4 stirred into 500 ml of 95% alcohol), and extracted 15 min. The residue was then boiled 1 hr with 5 ml of water and water extracted 15 min. The combined aqueous starch solutions were then treated in exactly the same manner as described for the 80% alcohol extract, except that acid hydrolysis was continued for 1 hr, and only 100 mg of carrier glucose were needed to assure satisfactory crystallization.

There were obtained from the starch extract 69 mg of crystalline glucose, 0.25 μc/mg, and from the soluble sugar extract 189 mg, 0.027 μc/mg.[3] Of the total activity assimilated, 26% was accounted for in the starch and soluble sugar extracts, 11% in the $BaSO_4$ residue from the alcohol extract, and 32% in the residual leaf material.

The sweet potato was used because of the ease of maintaining a continuous leaf supply for preliminary work. For the specific purpose of glucose preparation, other leaves are apparently superior. Whereas the potato leaf fixed 26% of the assimilated activity as starch and soluble sugar, Aronoff *et al.* (using the barley seedling) report 25 to 35%, Putnam *et al.* (using the tobacco leaf) apparently report 45 to 55%, and Livingston and Medes (using the bean leaf) claim 90% fixation.

Significant improvements will now be summarized: (1) A compact and simplified photosynthesis vessel permits considerable economy of time and effort in manipulation. (2) No special devices or leaf pretreatment are necessary to preserve humidity or the water content of the leaf, as evidenced by the fresh turgid condition of the leaf after a 24-hr photosynthesis period and by the essentially quantitative assimilation of a substantial amount of CO_2 during this period. (3) No special temperature control apparatus is necessary, despite the proximity of light source and leaf. (4) The evolution of CO_2 within the vessel is rapid yet under positive control, eliminating the spattering hazard.

Perhaps the greatest advantage is the adaptability of the apparatus to subsequent fractionation procedures. For example, the leaf may be directly homogenized in the photosynthesis tube by the insertion of a rotating pestle, after the manner of Potter and Elvehjem (*5*). Centrifugation or, with the use of the universal microapparatus to be described elsewhere (*3*), filtration and extraction procedures are possible without transfer of the plant material. Any such minimization of transfer is highly desirable when dealing with small amounts of material, especially radioactive material.

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[3] These are approximate values as based on the stated activity of a $BaC^{14}O_3$ shipment from Oakridge.