737. *An Apparatus for Preparative Photosynthesis of ^{14}C-Labelled Substances.*

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An apparatus is described which permits photo-synthesis on a laboratory preparative scale of systems useful for producing isotopically labelled substances. The biosynthesis of carbohydrate using tobacco leaves and of whole organisms using the alga *Chlorella vulgaris* is illustrated. The system is flexible, depends solely on artificial light, and allows efficient circulation of $^{14}CO_2$ in both gaseous and liquid systems.

In this paper is described an apparatus for the photosynthesis of carbohydrate, using isolated leaves, or for growth of whole organisms using algae. The application of the apparatus is described in the preceding paper. The apparatus makes use of artificial light and is thus independent of weather. Rates of synthesis are comparable with those obtainable by using sunlight.

Experimental

Use with isolated leaves.

Description.—The apparatus is designed for the brilliant illumination of tobacco leaves or algae, maintained at a uniform temperature in a continuously circulated atmosphere of air and radioactive carbon dioxide. The carbon dioxide is liberated from $Ba^{14}CO_3$ and provision is made for the recovery of any unassimilated activity. The radioactivity of the circulating atmosphere may be estimated continuously throughout the run.

Fig. 1 illustrates the gas circuit, and Fig. 2 the water-cooling and lighting systems.

For use with isolated leaves only the leaf chamber, *A*, and that portion of the assembly to the right of it in Fig. 1, are used. The chamber, *A*, consists of a 7 l. glass cylinder with ground flanges at each end. The lower end is closed by a removable ground-glass plate held by clamps, the upper end is closed by a desiccator top with a B34 socket. Two wires carrying the mains electricity supply to the pump are sealed into the corresponding B34 cone which also carries an inlet and outlet for the circulating gas and a socket for a thermometer. The pump, *P*, is a small A.C. vibratory model (J. W. Towers, Widnes) primarily designed for the aeration of fish tanks. A Geiger–Müller tube is mounted in a "counting chamber," *B*, the tube being held by four greased rubber rings in the upper half of a bell-shaped black-painted glass chamber. This chamber has a ground flange at the lower end and is sealed by a ground glass plate carrying inlet and outlet tubes. The rubber rings provide an adequate gas seal but allow the Geiger tube to be moved up and down, thus varying the sensitivity of the counting unit. The sensitivity can be further reduced for high-activity work by the application of a coat of cellulose paint to all but the centre portion of the mica window [this paint can be conveniently removed with methyl cellosolve (2-methoxyethanol)]. Water contained in the humid air leaving the leaf chamber is removed from the circulating air stream by the condenser mounted on the inlet side of the counting chamber. If this is omitted condensation occurs on the counter window.

The funnel, *C*, contains lactic acid which may be run on to the $Ba^{14}CO_3$ contained in the liberation chamber, *D*, attached by a spherical ground-glass joint. Traps 1 and 2 of the gas washing unit, between taps T_5 and T_7, are filled with saturated barium hydroxide solution. Trap 3 contains 40% sodium hydroxide solution. Taps T_1—T_8 serve to isolate various parts of the apparatus and their use is described below. The gas circuit is completed by the simple manometer, *E*.

The spiral growth chamber, *H*, illustrated in Fig. 1, is only used for photosyntheses with algae. When leaves are used the gas circuit runs directly from leaf chamber to the condenser.

The leaf chamber, *A*, stands in a Perspex cylinder, 25 cm. in diameter (Fig. 2), which serves as a water-jacket. Cooling water enters the lower inlet, *F*, from a constant-head reservoir and leaves to the sink by the upper outlet, *G*. The chamber is warmed by the lighting system, and constant temperatures in the range 20—40° can be maintained by adjusting the height of the constant-level reservoir and thereby altering the rate of flow of cooling water.

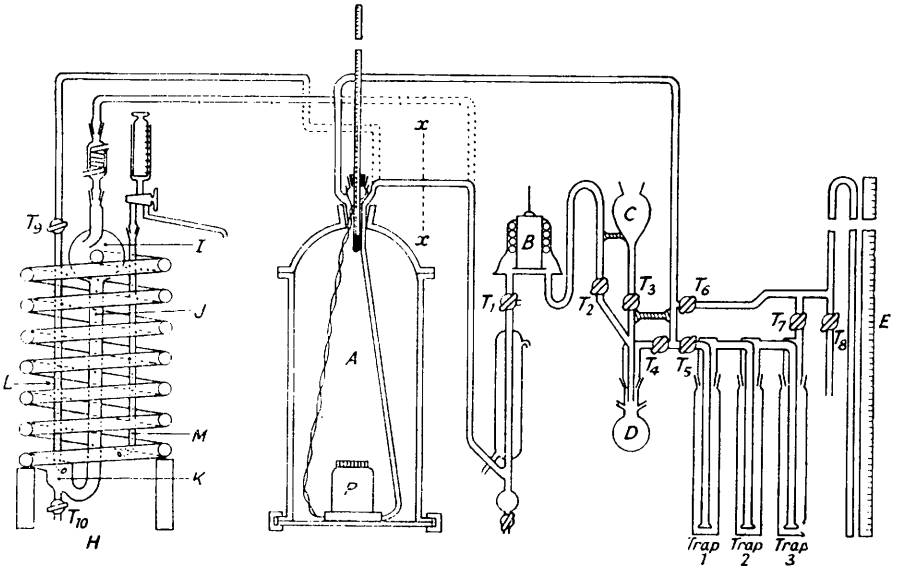
Light is supplied by sixteen 30 w Osram "Striplites," each 28·4 cm. long. Four are mounted on each of four quarter-cylindrical metal reflectors which together form a complete cylinder 40 cm. in diameter. The distance from the lamps to the surface of the leaf chamber is

10 cm. An approximately uniform illumination of about 3000 metre candles in the photosynthesis chamber is thereby obtained. It is necessary to cool the lamps with the fan illustrated (Fig. 2), to prolong their life in the confined space.

Operation.—Three or four leaves from a starved tobacco plant [1] are placed in separate 50 ml. beakers on the floor of the leaf chamber. Water (20 ml.) is added to each beaker and a wire frame is inserted to prevent the leaves from falling inwards.

A weighed quantity of $Ba^{14}CO_3$ containing the desired activity is placed in the liberation chamber. The counting chamber and the gas-washing unit are isolated by closing taps T_1, T_2, and T_5, T_7 respectively. The three-way tap T_1 leaves the counting chamber open to the atmosphere, thus protecting the delicate window should any leak at T_1 or T_2 develop during the evacuation of the apparatus. The apparatus is then evacuated through T_8 to a pressure of about 250 mm. mercury, and T_8 is closed. The manometer is read and checked again after 10 min. for leaks. An excess of *ca.* 50% (v/v) lactic acid is then admitted through T_3 during 10 min. The spherical ground-glass joint of the liberation chamber allows the contents of the chamber to be agitated should any unwetted $Ba^{14}CO_3$ be deposited on the walls above the level of the acid during the initial effervescence.

FIG. 1. *The gas circuit. For explanation of lettering, see text.*



Complete evolution of carbon dioxide takes about 30 min. Air is then admitted through T_8 to a final pressure 100 mm. below atmospheric, then T_8 and T_6 are closed. After slight expansion of the internal atmosphere when the apparatus is warmed to 30° there is still a slight pressure differential opposing the escape of $^{14}CO_2$ should a leak develop.

Taps T_1 and T_2 are turned to bring the counting chamber into the gas circuit, and the pump is turned on. The internal atmosphere is thus drawn into the pump and circulated rapidly past the counting chamber, through the liberation chamber, and then back into the top of the leaf chamber. The initial counting rate is measured and the lights are switched on. The circulating activity is measured continuously and readings may be taken at suitable intervals. The lights are turned off when the activity falls to background level. The counting chamber is again isolated and the apparatus evacuated slowly through the gas-washing unit. No precipitate is observed in the barium hydroxide traps after a successful run and this step may thus be omitted if the activity of the circulating atmosphere has fallen to background level. The system is opened to the atmosphere, and the leaves are removed for immediate working up.

Use with Chlorella.

Description.—The apparatus provides for the adequate illumination, aeration, and agitation of the medium and for the temperature control necessary for the rapid growth of *Chlorella* of high nitrogen content.

[1] Cf. Dalgliesh and Dutton, preceding paper.

The apparatus used for synthesis with tobacco leaves is used in its entirety. The original leaf chamber is now used as a gas reservoir and no longer stands in the illuminated water-bath. This position is instead occupied by the spiral growth chamber, *H* (Fig. 1), which is inserted in the gas circuit between the leaf chamber, *A*, and the counting chamber, *B*. The circuit used in the leaf experiments is broken along the line *x–x*, and the spiral connected into the circuit as shown by the broken lines in Fig. 1. The spiral of the growth chamber has an external diameter of 20 cm. and is 30 cm. high. The internal diameter of the tube is 1·6 cm. and the total capacity 950 ml. The top of the spiral opens into the side of a 250 ml. chamber, *I*. A vertical tube, *J*, leads from the bottom of this chamber to a smaller chamber, *K*, at the lower end of the spiral. The circulating gas is led into this lower chamber by the tube, *L*, and leaves *via* the splash-head mounted in the top of the upper chamber. A small water-cooled condenser is mounted immediately above the outlet. The vertical tube, *M*, opens into the spiral near its base and is surmounted by a detachable 10 ml. syringe which allows a sample to be withdrawn from the spiral *via* a two-way diagonal tap. The spiral growth chamber stands on a Perspex ring and, when it is in position in the bath, the outer surface of the spiral is 10 cm. from the ring of lights. The rest of the apparatus is as described above.

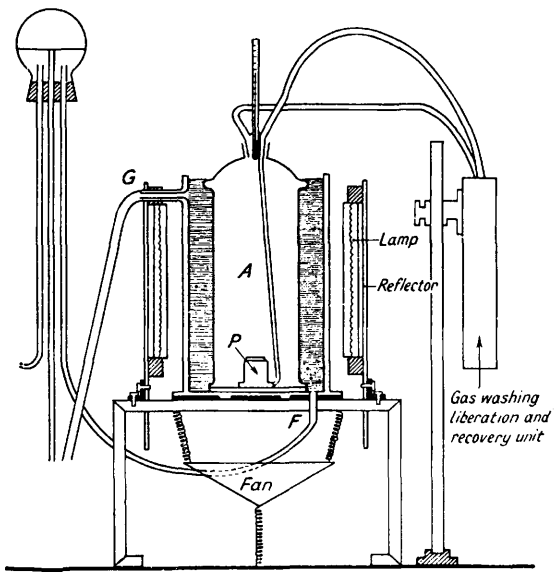


FIG. 2. *A cross-section of the apparatus showing the cooling and lighting systems, in this case the leaf chamber being in the water-bath. For explanation of lettering, see text.*

The circulating gas from the " leaf-chamber " reservoir enters the spiral at the lower chamber, *K*. From here bubbles ascend the length of the spiral, carrying pockets of medium before them. When the upper chamber is reached the gas escapes, to return to the counting chamber *via* the two condensers, while the medium descends again to the lower chamber through the vertical tube, *J*. By means of this " lift-pump " action, the medium is quite rapidly circulated up the spiral and back down the central tube. As the bubbles move up the spiral, the suspension of growing *Chlorella* is flattened out in a thin film around the inside of the tube and considerable local stirring is effected.

Operation.—The spiral is partially filled with 650 ml. of medium inoculated with 10—30 mg. of *Chlorella vulgaris*. The operation of the apparatus is much as described for the leaf syntheses. It is necessary, however, to close T_9 during the evacuation of the apparatus to prevent the medium from being sucked back through the gas inlet tube. The $^{14}CO_2$ is liberated and the lights are turned on. Samples of the growing *Chlorella* suspension may be withdrawn for turbidimetric or direct dry-weight determinations, by means of the syringe, while the activity in the circulating atmosphere can be measured continuously. The lights are turned off when dry-weight and activity measurements indicate that all the carbon dioxide has been utilised. The uptake of carbon dioxide is virtually quantitative and it is unnecessary to recover residual activity. The spiral is uncoupled and removed from the water-bath, and the dense suspension of *Chlorella* drained from the spiral through the tap T_{10}.

Results and Discussion

Tobacco Leaves.—The uptake of carbon dioxide by tobacco leaves is illustrated in Fig. 3, in which the activity in the circulating atmosphere is plotted against time. After a lag period, the rate of uptake becomes linear. The delay before linearity is established is due to two causes: (*a*) the liberation of carbon dioxide and homogenisation of the internal atmosphere is not instantaneous, and (*b*) the internal temperature rises after the lights are switched on, to reach an equilibrium temperature, conveniently about 30°. If the water-bath is initially at 30° and the lights are not switched on until the activity in the circulating atmosphere is constant, the uptake curve is linear from the beginning, but for preparative purposes liberation of carbon dioxide and illumination can be started at or about the same time.

The rate of uptake depends on the condition and the surface area of the leaves over the greater part of the carbon dioxide concentration range used. If these two factors are kept constant, and the number of lights is halved, the rate of uptake is also halved.

FIG. 3. *Uptake of* CO_2 *by tobacco leaves.* $^{14}CO_2$ *was liberated from* 1·54 *g. of* $Ba^{14}CO_3$. *Three leaves, of surface area* 780 *sq. cm., together weighing* 26 *g., were used and the mean rate of uptake was* 12·6 *mg. of* CO_2 *per dm.*^{2} *per hr.*

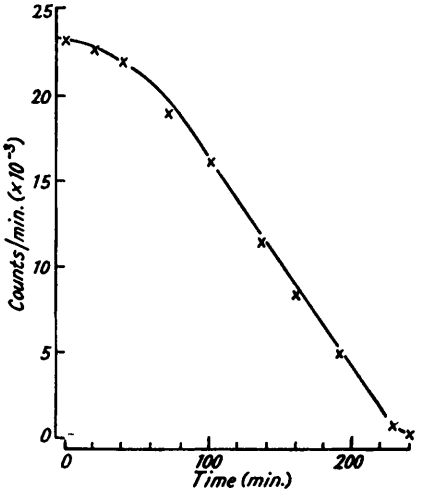
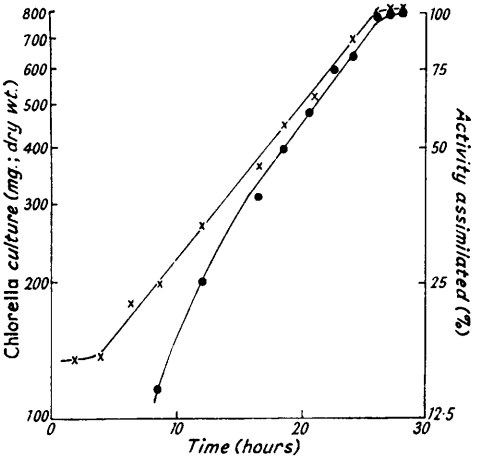


FIG. 4. *Growth of a* Chlorella vulgaris *suspension. The growth curve* (×——×), *and the percentage of circulating* $^{14}CO_2$ *assimilated* (●——●), *are plotted on a logarithmic scale, with* 100% *uptake equated to the maximum dry weight of algae obtained.*



The intensity of illumination is the rate-limiting factor. Under normal working conditions the carbon dioxide content of the circulating atmosphere is initially high (8% if carbon dioxide from 5 g. of barium carbonate is liberated) but although such a concentration would normally be considered toxic it here appears to have no deleterious effect. Similar high initial carbon dioxide concentrations were without toxic effect in the experiments of Putman *et al.*[2] and Porter and Martin.[3] The uptake of carbon dioxide is normally complete, as judged by the absence of precipitate when the internal atmosphere is drawn through the barium hydroxide traps at the end of the experiment. However, leaves from plants raised in mainly artificial light during the winter months were less satisfactory than summer-grown material and occasionally failed to take up carbon dioxide at all.

In a series of seven experiments weights of $Ba^{14}CO_3$ ranging from 1 to 5 g. were used containing up to 9 mc of ^{14}C. Between 15 and 25 g. (fresh weight) of leaf were used in each run with an average surface area of 700 cm.^{2}. The time taken for assimilation varied from 3 to 7 hr. and the uptake was from 95 to 100%. The rate of uptake of carbon dioxide varied from 8 to 15·4 mg. per dm.^{2} of leaf area per hour, with a mean value of 12·2.

[2] Putman, Hassid, Krotkov, and Barker, *J. Biol. Chem.*, 1948, **173**, 785.
[3] Porter and Martin, *J. Exp. Bot.*, 1952, **3**, 326.

Algae.—The apparatus is designed for the production of algae intended for use as a source of ^{14}C-amino-acids. The nitrogen content of *Chlorella vulgaris* was found to vary from approximately 4% in resting (agar slope) cultures to 9% in cultures grown at the maximum growth rate. In this work it was therefore necessary to adopt conditions favouring the most rapid rates of growth. This can be achieved by using the spiral growth chamber described. Aeration of the medium is made very simple as the air stream is constrained to flow through the whole medium and for this a small pump is adequate. Further, the whole medium is rapidly circulated and a homogeneous suspension maintained.

The uptake of carbon dioxide, and algal growth, are illustrated in Fig. 4. The growth curve is logarithmic over the greater part of the range, which indicates that neither carbon dioxide concentration nor intensity of illumination is limiting under these conditions, as both these factors alter during the experiment (the effective intensity of illumination falling as the culture density increases). The rate of growth increases with rise in temperature. The apparatus is normally operated at 30°, the mean generation time being 10 hr. In each of a series of experiments the carbon dioxide was liberated from 5 g. of $Ba^{14}CO_3$ containing up to 27 mc. The carbon dioxide uptake was quantitative, and, after being washed and dried, the algae accounted for more than 92% of the carbon supplied. The synthesised *Chlorella* had a mean nitrogen content of 9%.

The two biosyntheses described above are only examples of possible applications of the apparatus, which is both flexible and versatile and is useful in many other problems of preparative photosynthesis.[4]

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[4] See, *e.g.*, Badenhuizen and Dutton, *Protoplasma*, 1956, in the press; *Biochem. J.*, 1956, **62**, 13P.