NOTES.

THE ACTION OF CHLOROFORM ON CO_2-ASSIMILATION. —In 1878, Claude Bernard[1] observed that chloroformed water-plants ceased to evolve bubbles of oxygen in the light but that they recovered this power if the chloroform was at once removed. In Schwarz's[2] experiments, however, the evolution of bubbles ceased only when the plants were fatally affected and had lost the power of recovery, and similar results were obtained by Pringsheim[3]. Bonnier and Mangin[4] found that in certain Phanerogams, by using measured quantities of ether, CO_2-assimilation might be stopped without the respiratory activity being affected, and I have recently[5] shown that the prolonged action of ether-vapour causes the chloroplastids to become temporarily or permanently inactive. Ether may therefore not only directly render CO_2-assimilation impossible, but may also finally induce a condition in the chloroplastid during which it is unable to assimilate even when returned to normal conditions. As regards the effect of chloroform further research was evidently necessary, especially since Kny states that filaments of *Spirogyra crassa* immersed for 5 hours in a mixture of one part of saturated chloroform-water to five parts of tap-water showed clear signs of death, but nevertheless were still capable of CO_2-assimilation[6].

Spirogyra nitida (?) was entirely killed after being kept for 8 hours in a saturated solution of chloroform in water, and no evolution of

[1] Claude Bernard, Leçons sur les phénomènes de la vie, 1878, p. 278.

[2] Schwarz, Bot. Unters. aus Tübingen, 1881, p. 102.

[3] Pringsheim, Sitzungsb. d. Akad. d. Wiss. zu Berlin, 1887: 'Die Abhängigkeit der Assimilation grüner Zellen von ihrer Sauerstoffathmung.'

[4] Bonnier and Mangin, Ann. Sci. Nat., Sér. vii, t. iii, 1886, p. 14.

[5] Ewart, Journ. Linn. Soc. Bot., Vol. xxxi, 1896, p. 408.

[6] Kny, Ber. d. D. Bot. Ges., 1897, Bd. xv, p. 401.

oxygen was observed from any of the cells even though the chlorophyllous contents retained an almost normal green colour. If kept for the same time in a semi-saturated solution of chloroform in water, many cells are still living and plasmolysable; but either no evolution of oxygen or in a few cases a faint or doubtful one is shown on examination. Next day a distinct to moderately active power of CO_2-assimilation is shown by living cells, but these are extremely few in number, most having died. Kny largely employed as test-bacteria those taken from putrescent fluids containing meat, and moreover did not consider that it was necessary to ring the preparations in order to exclude external oxygen. Under these conditions however there is a serious liability to error, for such fluids almost always contain facultative or partial anaerobes which continue to move in the absence of oxygen and are attracted by the nutritious substances exuded from dying cells. Such movement continues in the darkness though it may appear as if it recommenced immediately the preparation is exposed to light.

The same author also states that cells killed by acid and by the action of strong induction-currents might continue to assimilate carbonic acid, and in the latter case with an increased activity! A possible explanation of these results has already been given [1], and it seems almost incredible that so keen an observer as Kny could have been led into so palpable an error. The actual experimental work seems however to have been performed by Kny's [2] assistant, and it is hardly necessary to emphasize the fact that the delicate bacterium-method can only be trusted to yield accurate results when it is properly applied by a capable experimenter.

Elodea is a much more suitable plant for experimentation than *Spirogyra*; and if a plant of *Elodea* is kept for one day in a saturated watery solution of chloroform, all the cells are killed and no trace of an evolution of oxygen from them can be detected. A plant suddenly saturated with a watery solution of chloroform containing a slight excess of the latter in the form of a fine emulsion, becomes covered with gas-bubbles if exposed to sunlight, and from the cut end of the stem bubbles derived from CO_2-assimilation continue to escape actively for five minutes, then slowing and ceasing in the succeeding

[1] Bot. Cent.-bl. 1897, Bd. lxxii, No. 9 (Relations of Chloroplastid and Cytoplasma).

[2] Kny, l. c., p. 403. Bot. Cent.-bl., 1898, Bd. lxxiii, p. 439.

five minutes. The chloroform appears at first to exercise a physical action favouring the formation of gas-bubbles, and the heating effect of the sun's rays may also aid in causing an evolution of bubbles after the plant has been fatally injured, for after five minutes immersion the plants have lost the power of recovery and rapidly die in fresh water. By using more dilute proportions and longer periods of exposure it is possible to cause a cessation of CO_2-assimilation without the power of recovery being lost.

Elodea canadensis. (*a*) Plant exposed to sunlight in water at 18° C., super-saturated chloroform-water allowed to trickle slowly in, and the water gently agitated. Evolution of bubbles slows after ½ hour and ceases in 1 hour. Water rises to 28° C. in this time, and hence intercellular air (⅛ c. c.) is increased by ½ c. mm. in volume, an appreciable fraction of the total amount of gas evolved but probably partly compensated for by the increased respiratory activity. Leaves washed and immediately examined, show no rotation, no evolution of oxygen, and the chloroplastids do not take an apostrophic position in strong light. Next day the plant shows feeble evolution of gas-bubbles: on examination about half the leaf-cells and nearly all the stem-cells are living and show with the bacterium-method a moderately active evolution of oxygen, which in a few cases is feeble or doubtful.

(*b*) Experiment repeated with plant in water at 27° C., evolution of bubbles slows in 10–15 min. and ceases in 20–25. Plants immediately washed and placed in fresh water show no evolution of bubbles after 3 hours, but occasional bubbles after 5 hours; while a leaf when first examined shows no rotation, no evolution of O, and the chlorophyll-grains remain dispersed in strong light, in 3 hours there is faint to moderately active evolution of oxygen and the chloroplastids assume the apostrophic position. Next day moderately active evolution of bubbles is shown, and most of the leaf-cells remain living and show active rotation.

These results therefore confirm the original experiments by Cl. Bernard, and show that a stoppage of CO_2-assimilation may be produced by uniformly distributed anaesthetization if properly graduated to the resistant powers of the plant employed.

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