Botany. — "*A method of simultaneously studying the absorption of* O_2 *and the discharge of* CO_2 *in respiration.*" By D. S. FERNANDES. (Communicated by Prof. F. A. F. C. WENT.)

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Before entering into details, writer will briefly indicate, how the apparatus works and what precautions should be taken, illustrated by a simple diagram. (fig. 1).

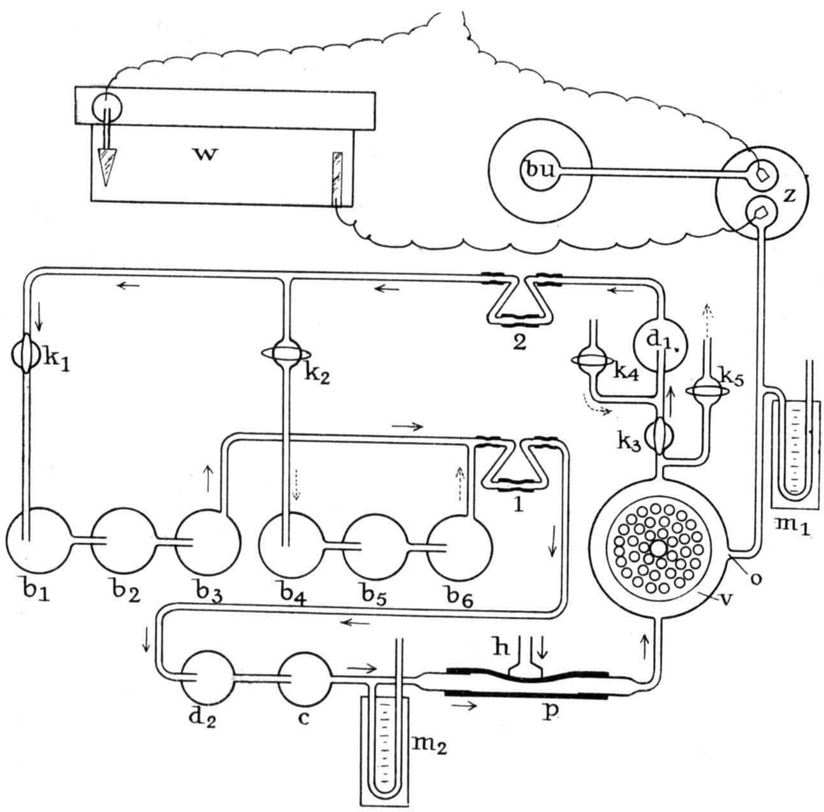

Fig. 1.

From p, a rubber sucking- and forcing pump, the air is pumped as the arrows indicate. The air enters the respiratory vessel v at the top, leaves it at the bottom and is dried in the wash-flask d_1, which contains concentrated sulfuric acid. From d_1 passing through the glass cock k_1 (k_2 is then closed) it reaches the absorptiontubes b_1, b_2 and b_3, containing baryta-water. On its way back the air passes through the wash-flask d_2, containing sulfuric acid like d_1 and the control-baryta-tube c, after which it returns to p and recommences its circular course.

In a subsequent observation k_1 is closed and k_2 opened, causing the CO_2 absorption to take place in the tubes b_4, b_5 and b_6. The 6 absorption-tubes are fixed to a copper frame with clips. In order to enable us to take more than two observations, without bringing too many tubes in the glass vessel filled with water, which serves as a thermostat, we should have two of these frames at our disposal. If one has served its purpose, the connecting parts 1 and 2 are turned up and rise above the water, where they may be loosened. The whole frame with the 6 baryta-tubes is raised out of the vessel and the other (the tubes of which are meanwhile cleaned and filled each with 100 c.c. baryta-water) is put in. This exchange of frames is brought about in less than a minute, but before taking further observations with the newly-inserted baryta-tubes, we should wait (according to the temperature in the thermostat) 10—15 mins. that the tubes and their contents may adopt the temperature of the thermostat. The apparatus works ventilating during this time in the following way: Cock k_3 is closed, while k_4 and k_5 are opened. If next the pump is set working, the air, leaving the vessel, can only pass through k_5, while at k_4 air is sucked in, after having first been rid of CO_2 by means of wash-flasks containing strong KOH-solutions (not represented in the fig.). There is another advantage in the ventilating action of the apparatus. When in experiments of long duration the observations are stopped in the evening, the apparatus can continue to work ventilating the whole night. Consequently the objects are not subject to oscillations of temperature and the next morning the experiment may at once be continued by opening k_3 and closing k_4 and k_5. In experiments, lasting 10—12 hours, it saves a great deal of time, to put the plants into the apparatus the previous night, so that early in the morning the experiments can begin at once. After the ventilation during the night all CO_2 has been driven from the apparatus which may be demonstrated by blind experiments.

When the outer-air is shut from the apparatus, and the pump is set working, there is immediately produced an effective pressure on the vessel, while the manometer m_2, indicates a reduction of pressure. If next k_5 is opened, the air pressed in the vessel is blown off. On subsequent gradual closure of this cock, the pressure in the vessel

$=1$. In the manometer m_1 the liquid is equally high in both limbs, whereas m_2 indicates a greater negative pressure than before. The broken equilibrium, generated by the action of the sucking-and forcing pump in the closed system is apparently shifted by the opening and closing of k_5 in such a way, that in the respiratory-vessel (accordingly on the plants) no effective pressure can arise. As soon as there disappears O_2 from the closed system through respiration, m_1 will indicate it at once. When however an equal quantity of O_2 is added at the same time, m_1 will remain at zero and the atmospheric pressure is preserved in the vessel. At O the oxygen, electrolytically produced in Z, enters the vessel. With the aid of the resistance w the O_2-development can be increased from a minimum to a definite maximum. The intensity of the electrolytic process may be thus regulated, that the O_2-production keeps pace with the O_2-consumption.

By increasing or reducing the resistance this equilibrium is soon found and the manometer m_1 indicates whether this condition is preserved. It may happen (for instance by rise or fall of the respiration-intensity), that for a moment there is a somewhat greater or smaller supply of O_2 to the apparatus. In this case the height of the manometer m_1, indicating as slight a difference as 0.1 cc., may at once be restored by means of the resistance, so that irregularities in the O_2 supply, amounting to more than 0,1 cc. need not occur.

The hydrogen simultaneously produced by the electrolysis in Z is collected in the burette bu. After necessary corrections (in height of barometer, temperature, water-vapour tension and pressure of the water-column in the burette) the quantity of hydrogen received, divided by 2, denotes the volume O_2, brought into the apparatus during the observation.

The manometer m_1 renders some other services. When a solution of kalium-jodide (with some soluble amylum) is used, m_1 is a sensitive test for the existence of spores of ozon. In the presence of this gas for instance the germ-plants of Pisum sativum do not develop normally, so that it is desirable to prevent ozon from entering the respiratory-apparatus.

Finally we have in the manometer m_1 a suitable test whether the desired temperature has been completely adopted by the whole apparatus as well as by the objects. If the observations are started before the whole has attained the desired temperature, the fluid will at once rise in the open limb of m_1, which signifies, that extension still takes place, while in consequence of the respiration (O_2 absorption) an immediate decrease of volume should appear.

For determining the period of preheating therefore m_1 is of practical interest.

The watervapour carried along from the vessel is combined in d_1 so that dry air enters the baryta-tubes. The watervapour taken from the lye is absorbed in d_2. By measuring the increase of volume in d_2 it may be found, how much water disappears from the lye and the titration standard may be corrected accordingly. This evaporation from the baryta-tubes is very slight and amounted to circa 2 cc. in experiments lasting 3 days, so that the correction may be left out without scruple.

The manometer m_2 is filled with mercury and serves to indicate the pressure, to be surmounted by the sucking and forcing pump, needed to drive the air through the various liquids. A drop of paraffine-oil on the mercury in the closed limb, prevents the originating of damaging mercury-vapours.

On the rubber-pump p taps a flat hammer h, moved vertically by an electro-motor (not represented in the figure). This hammer may be mounted higher or lower in order to regulate the capacity of the pump and consequently the size of the bubbles. The speed of the motor may be increased or decreased by means of a resistance, with which the regulation of the number of bubbles is possible. Size and number of bubbles are of course material to a good CO_2-absorption.

For an equable distribution of the air, entering the vessel, the ebonite plates on which the plants lie, are brought into a slow rotary movement by an axis. Accumulation of CO_2 in the vessel (see further on) is excluded in this way.

The suction of the air into and from the vessel, causes the liquid in m_1 to move up and down, which is not to be prevented. At an effective regulation of the pump this movement may be kept so slight, that it is no impediment. Indeed the motor may be stopped at any moment, to convince oneself whether the manometer is really at zero.

The whole apparatus is fixed to the inside of a copper frame and fits exactly in a glass vessel (contents about 45 L.), serving as a water-thermostat. Electrical heating enables us to keep the temperature of the water constant to 0.03° C. The oscillations of temperature in the apparatus itself are slighter than those in the thermostat, so that corrections relating to this, may be omitted.

If the apparatus is immersed in the water of the thermostat, it may be easily tested with respect to air-tightness. For this purpose air is pumped into the apparatus through k_4 and one watches whether any bubbles rise from the water. When the connections are made with vacuum rubber-tube and glass to glass, leakages do not occur.

II.

Descriptions of the parts.

a. Sucking- and forcing-pump (fig. 2).

An air-tight pump, working for a long period without failing and having a sufficient capacity, is easily constructed.

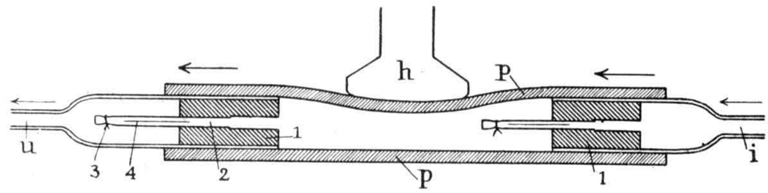

Fig. 2.

The glass tubes i and u are connected by a piece of strong rubber-tube p (about 15 cms. long and $2^1/_2$ cms. wide). Each of the tubes i and u is provided with a valve, consisting of a piece of vacuum-tube (1 cm. long) 1, to which the end of a piece of valve-tube 2 (about 3 cms. long) is glued on with solution. The other end of the valve-tube is tightly tied with a string at 3; in the valve-tube a straight lengthwise cut 4 is made, the two edges of which meet, when the pump does not work. To prevent these edges from sticking together afterwards, they have been rubbed in with talcum powder. The glass tubes i and u fit in the rubber-tube p, while the vacuumpieces 1 must also fit perfectly. How the pump works, when the hammer h taps on it, is clear from the fig. 2.

b. The respiratory-vessel (fig. 3).

As in Kuyper's research[1]) here too is made use of a copper cylinder 1. The experimental objects are on the ebonite plates t, fixed to an axis a_2. In each of the plates t 25 round holes are made in such a way, that germinating seeds of Pisum sativum cannot fall through. On the plates t_1 moist cotton-wool is put, on which the roots rest, in consequence of which there cannot occur a deficiency of water. The axis a_2 is enlarged at the top, provided

[1]) Kuyper J: Recueil des Travaux Botaniques Néerlandais. Vol. VII. 1910, pag. 1.

with 4 teeth *ta*, just fitting into the four teeth ta_1 belonging to a simular enlargement at the base of the axis a_1. This steel axis a_1 passes through a copper case k (soldered to the cover), in which it

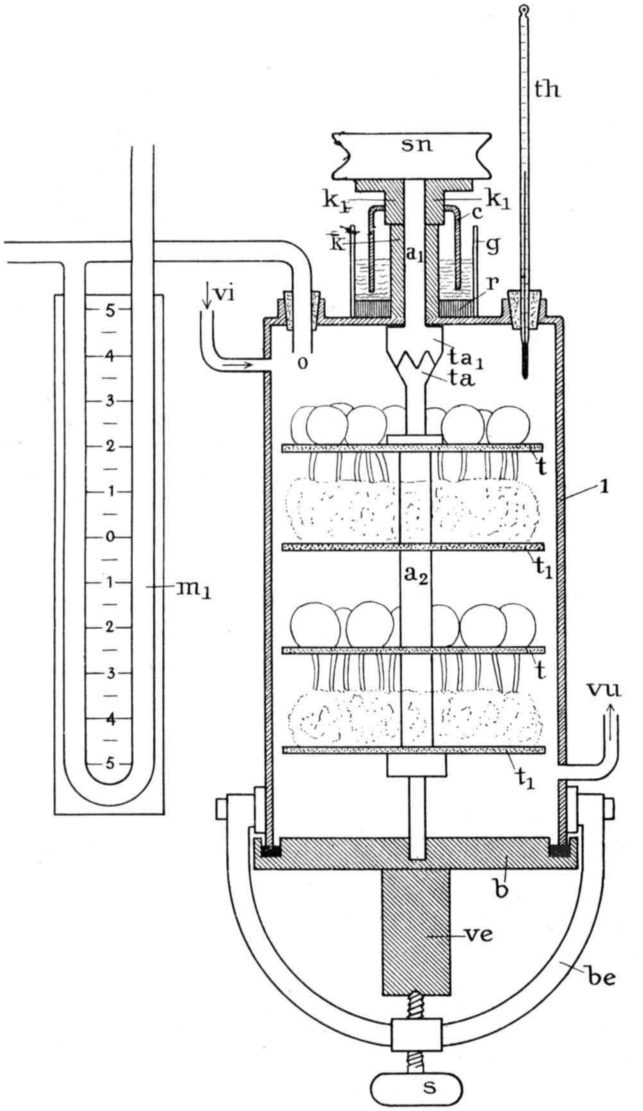

Fig. 3.

fits exactly, but may be easily rotated. Round k there is a glass cylinder g, closed at the bottom by the india-rubber-ring r. The axis a_1 is at the top tightly clasped in a copper tube k_1, at the bottom of which the hollow metal cylinder c is fastened, and at the top the grooved wheel *sn*. By the oil in g the axis is closed off air-tight and leakage is impossible, because there never arise great differences of pressure in the vessel. In the middle of the loose part *b* there is a cavity, in which a_2 can rotate freely. When *sn* is slowly rotated by a motor, a_1 will transmit this movement by means of the teeth ta_1 and *ta* to a_2, which causes the circulating air to be equably distributed over the whole vessel, in consequence of which the germplants are constantly surrounded by fresh air. The necessity of ventilation in a cylindrical respiratory vessel (diameter 15 cms., height 20 cms.) was immediately apparent from one of the many test experiments. At a constant temperature of 20° C. the O_2-absorption caused in 50 mins. a height of 4 cms. on the manometer m_1. Next a quicker circulation of 10 mins. duration followed, causing an equal rise of the manometer as before in 50 mins. No other explanation of this could be found, but the occurrence of a CO_2-accumulation in the vessel. This was supposed to be due to the fact, that the air entering at *vi* passed by the easiest route through the vessel to the exit *vu*, taking with it only part of the CO_2. When in consequence of a more rapid circulation part of the accumulated CO_2 disappeared, this explained a sudden greater rise of the manometer. As soon as the rotary movement of the respiring objects, prevented all CO_2-accumulation in the vessel, there was indeed no abnormal rise of the manometer to be noticed. It needs no argument, that not only with a view to oxygen-supply and measurement, but also for other reasons, the CO_2 due to respiration, should be directly removed. With a CO_2-accumulation in the vessel, a volumetric determination of the vanished quantity of O_2 is no more possible. Besides in this case part of the plants gets into an atmosphere full of CO_2 and deficiency of O_2 will soon cause intramolecular respiration.

It seems to me, that in the respiratory apparatus after the model given by PFEFFER and DETMER and used e.g. by KUYPER, little or no attention has been paid to the error which may be committed, when in a respiratory vessel as described in this paper, no perfect ventilation is provided for.

The loose bottom *b* is provided with a marginal groove, containing a rubber-ring. The handle *be* bears in its middle a screw *s*, which, when turned up, presses on *ve* and by doing so presses the lower edge of the vessel tightly in the groove with rubber-ring.

In the cover of the vessel is, besides the aperture *o* to admit oxygen, also a pierced rubber-cork through which a thermometer *th* passes.

c. Fig. 4 gives a representation of the *drying-tubes* and the *control-tube.* Cock 1 serves for filling, cock 2 for emptying and cleaning.

d. The *absorption-tubes* are fastened to a copper frame (fig. 5). As with a view to preversing a constant temperature the size of the thermostat cannot be chosen at will, straight absorption-tubes (length 25 cms., width 3 cms.) are more suitable than PETTENKOFER or WINKLER-tubes. When baryta-water is chosen for combining with CO_2 (21 grammes of bariumhydroxyde + 3 grammes of bariumchloride in 1 L. of water), the absorption is only complete, when the air passes through 3 of those tubes (each containing 100 cc. lye). Each frame of 6 tubes therefore can only serve for two observations. The tubes end at the base in thin open pieces, which may be plugged by rubber stoppers. At the top they are closed by rubber-corks 3 cms. thick. In each cork there are three holes, two of which serve for the inlet- and exhaust-tubes, while the third, which serves for filling can be plugged by a little massive glass bar. The tubes are connected with vacuum-rubber

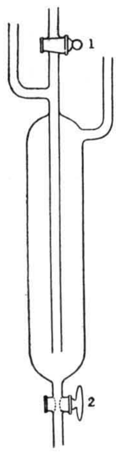

Fig. 4.

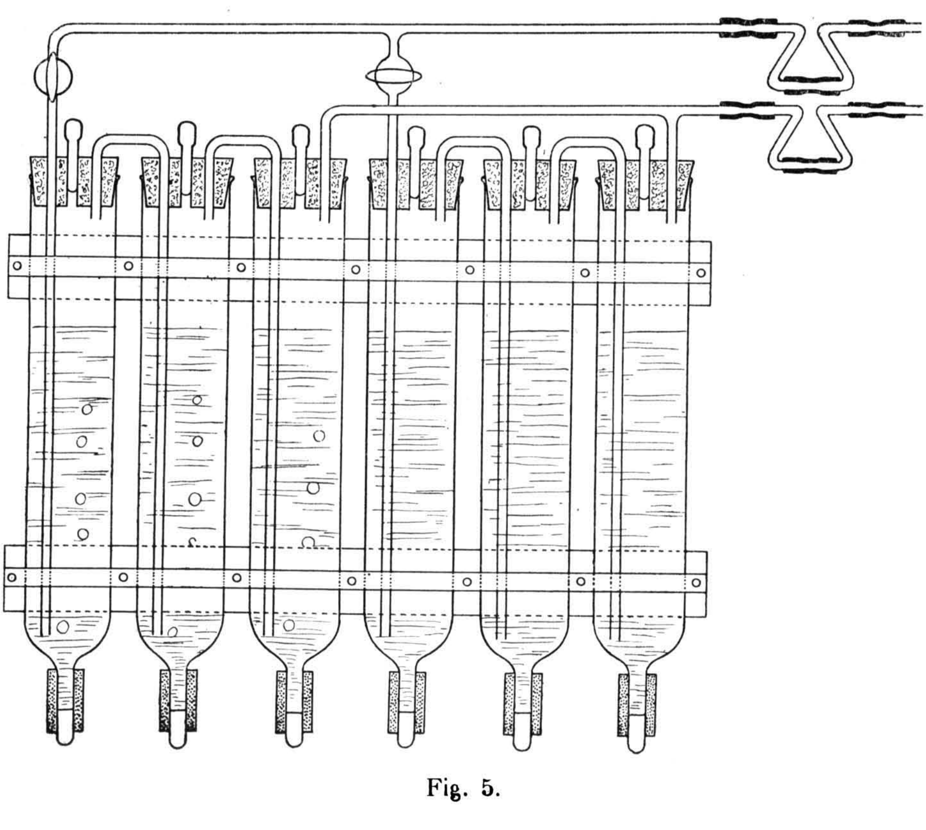

Fig. 5.

tube, just as all other connections in the apparatus are made. There was no sign of any CO_2 diffusion inward from the water of the thermostat through the rubber-connections and corks, nor of an O_2-absorption through the rubber. Blind experiments, lasting 24 hours gave no measurable change of titration standard of the lye at temperatures between 20° and 30° C., while the manometer m_1 remained at zero throughout that time.

e. The oxygen-supply and measurement.

In order to prevent ozon-formation, a 10 % natronsolution is to be preferred to diluted sulfuric acid for the electrolysis.

In fig. 6 C is a glass cylinder with natron-lye in which the platina-electrodes p_1 and p_2 are placed. By means of thin platina-wire these electrodes are fastened by melting in the glass-tubes 1 and 2 respectively. The tubes 1 and 2 pass through caoutchouc-

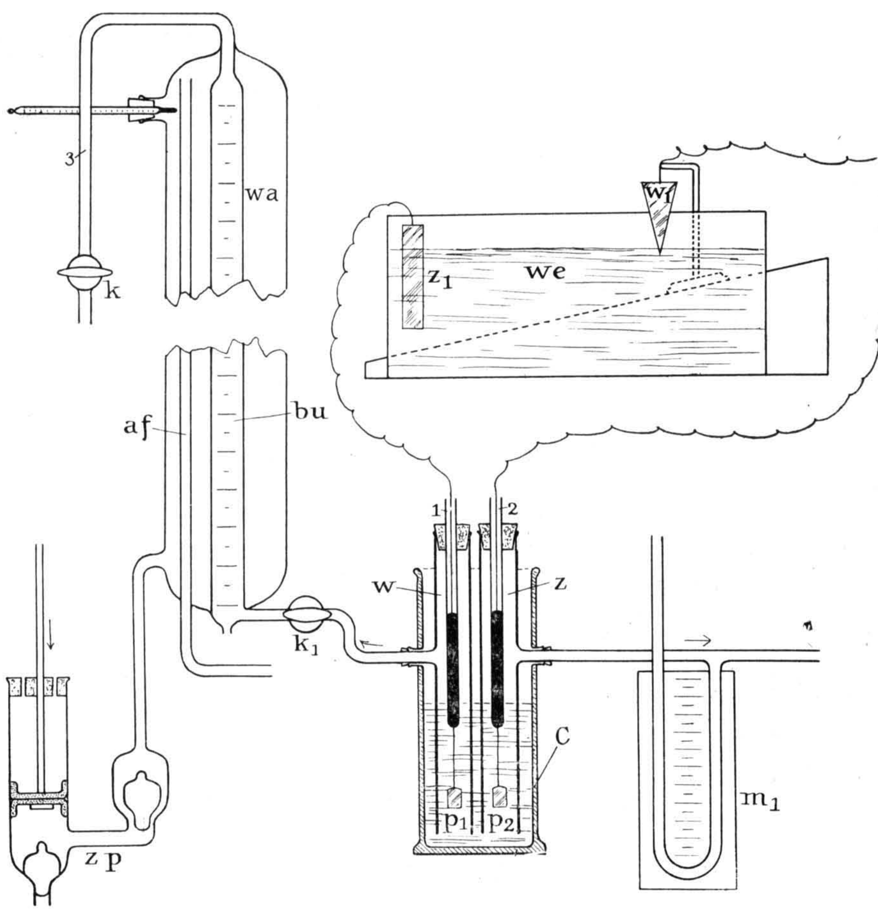

Fig. 6.

corks, fitting exactly in the wider tubes *w* and *z* (open at the bottom) and are filled with some mercury. By means of a resistance *we* the intensity of the current can thus be regulated, that the amount of the electrolysis can reach the desired extent. Thus it is possible to keep the oxygen-development, occurring in the tube *z* at the electrode p_2, in balance with the O_2-consumption of the respiration. As a resistance (*we*) a glass basin with water, in which the electrodes w_1 and z_1, is quite satisfactory for this purpose. By moving w_1, which is fastened to a stand, along a sloping board, not only the distance w_1—z_1 is made smaller or larger, but this electrode also goes more or less deep in the water.

The O_2 formed in *z* is in open connection with the manometer m_1 and the respiratory-vessel. The tube *z* really is likewise a manometer, in which the lye will be equally high as in *c*, when the quantity of O_2 developed is equal to the quantity disappearing in the apparatus; m_1 however, as already mentioned, is necessary to control the ozon-formation.

For receiving the hydrogen, formed at the electrode p_1 in the tube *w*, the burette *bu* serves, which gives accurate readings to 0.1 cc. This burette ends at the top in a bent glass tube 3, provided with a glass cock *k*. At the bottom the burette has a narrow aperture, while not far from this a lateral tube has been fitted on, forming a connection with the tube *w*. When the burette is placed in such a way, that the bottom aperture lies just below the water-level in the thermostat, it is impossible, that while water is flowing out, air is ascending in the burette at the same time. Filling the burette with water from the thermostat is done by closing k_1, opening *k* and sucking at the tube 3. When after filling *k* is closed and k_1 open, the only reason why water should flow from the burette, is the formation of hydrogen in *w*, which rises in the full burette as bubbles. The formation of the first hydrogen-bubbles in the burette requires a little effective pressure, which is shown by the fall of the fluid in the tube *w*. This effective pressure, which remains constant during the emptying of the burette, should exist before the observations begin, lest the first reading should give a too small figure. This error is prevented, when some minutes before the experiment commences — when the apparatus still works ventilating — the electrolysis is made to take place, till the first bubbles rise in the burette. In case that, during one and the same observation, the burette is filled several times, the sucking up of the water should occur very slowly and equally, lest the hydrogen, which is in the connective-tube between k_1 and the burette, should be sucked in with it. If the water is sucked cautiously into the burette, the effective pressure once made is preserved in *w*.

Another error arises, when the burette is exposed to oscillations of temperature in the laboratory. In that case not only in *w*, but also in *z* and m_1 falls and rises occur, which are not due to absorption of oxygen. This may be prevented by keeping the burette likewise at a constant temperature, which may be attained as follows.

By means of a metal sucking- and forcing-pump *zp* (likewise fastened to the copper frame, to which the whole apparatus is fastened) water from the thermostat is pumped up with great rapidity into a wide glass cylinder *wa*, which contains the burette. The water enters *wa* at the bottom and is led back to the thermostat at the top through the tube *af*. Even at high temperature (50°, 55° C.), the temperature in the burette is kept equal to that of the water in the thermostat in this way.

f. The regulation of the temperature principally corresponds to the one described by RUTGERS[1]) and COHEN STUART[2]) and is an imitation of apparatus, used in the VAN 'T HOFF-laboratory at Utrecht.

The heating-apparatus *v* (fig. 7) consists of a copper case, surmounted by a metal tube, rising above water. In *v* is paraffine-oil, electrically heated by a nickel-chrome-wire, wrapped round a piece of mica.

Thermoregulator *t*, stirring-apparatus *r* and *v*, are close together in an open glass cylinder *c*, resting on legs in the centre of the thermostat *g*. To prevent all influence of vibration in the height of the mercury, the thermoregulator is hung from the ceiling on a steel spiral-spring, according to the method MOLL.

The method described above gives no new principle, with respect to the CO_2-determination. We have chosen the simple and always trustworthy baryta-method, which need not be further described here. On account of the insertion into a closed system, the various parts were subjected to some alterations in shape, which however have nothing to do with the principle of the baryta-method.

The problem of oxygen-supply, ever yielding many difficulties, could be satisfactorily solved. Compared with the methods[3]) already existing, the following advantages and simplifications are achieved:

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[1]) RUTGERS, A. A. L., Recueil des Travaux Botaniques Néerlandais. Vol. IX, 1912, pag. 1.

[2]) COHEN STUART, Recueil des Travaux Botaniques Néerlandais. Vol. XIX, Livraison 2. 1922.

[3]) Cf. KROGH: "The respiration exchange of animals and man. LONGMANS, GREEN and Co., London 1916".

a. the decrease of pressure and oxygen-content in the apparatus is reduced to a minimum.

b. the place of the consumed O_2 is at once taken by pure O_2, without first passing a stop-valve, and may directly be controled.

c. an oxygen-bomb or other reservoir may be omitted.

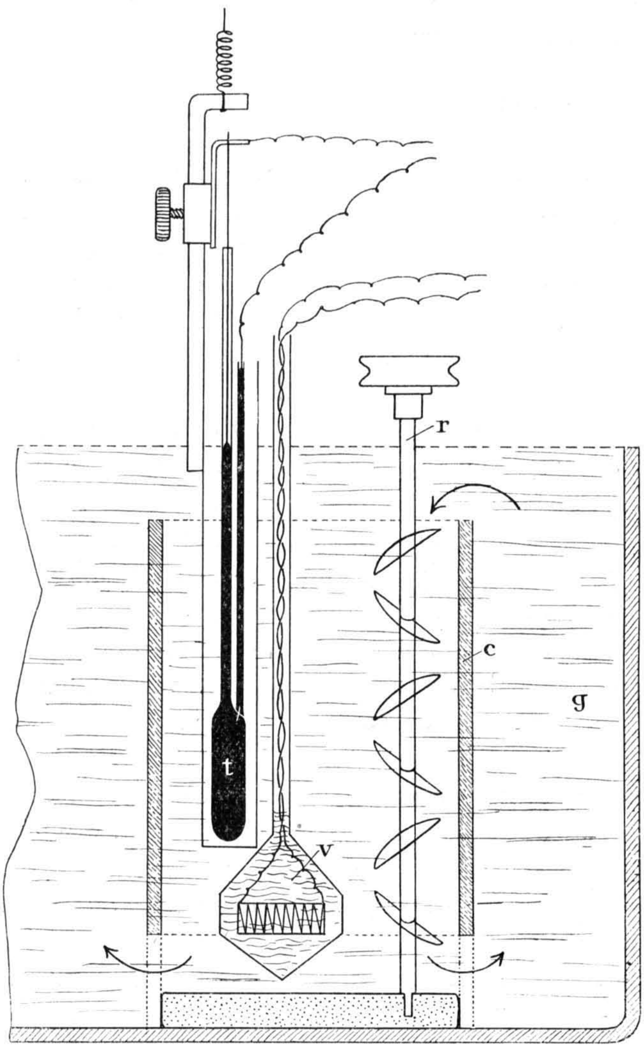

Fig. 7.

The apparatus has been constructed by Mr. P. A. DE BOUTER, amanuensis at the Botanical Laboratory at Utrecht. I am greatly indebted to him, not only for the way, in which he performed his task, but also for introducing some clever improvements.

Utrecht, May **1923**. *Botanical Laboratory.*