A SIMPLE RESPIROMETER FOR QUANTITATIVE CLASS EXPERIMENTS

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It is desirable that students in plant physiology be able to verify experimentally some of the known facts concerning respiration. In order to meet this need, a simple and inexpensive respirometer was devised for following the respiration of germinating seeds over rather short periods of time. This device is based upon the well known principle of determining the oxygen absorbed in respiration by manometric measurements of the resulting reduced air pressures. The carbon dioxide evolved is immediately absorbed by a sodium hydroxide solution so that the production of this gas has no effect upon the pressure of the atmosphere within the apparatus.

The respirometer consists of a large test tube (2.5 x 20. cm.) fitted with a two-holed rubber stopper, a U-shaped capillary-tube mercury manometer, a short glass rod about 3 cm. long and a small perforated cardboard disk. The arrangement of the apparatus may be seen in the accompanying diagram (Fig. 1).

In setting up an experiment, 5 c.c. of a strong solution of sodium hydroxide is first run into the bottom of the test tube from a pipette. Following this the

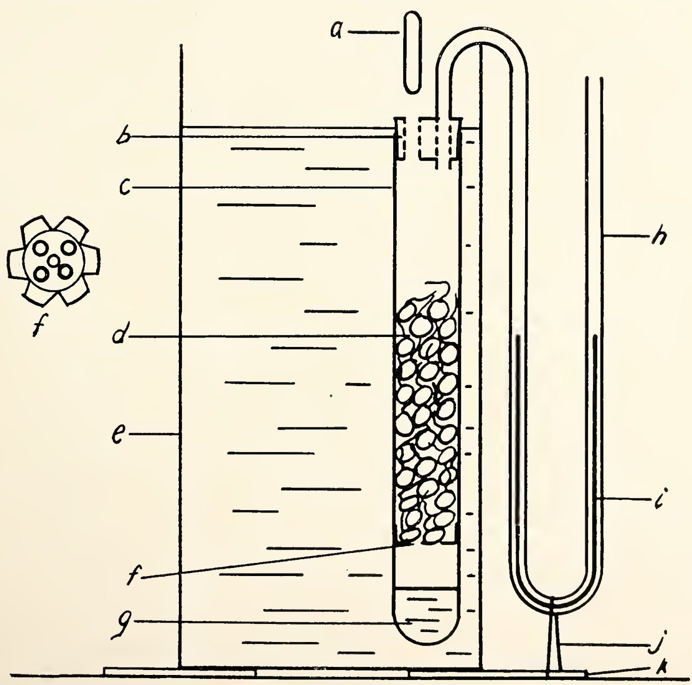

Fig. 1—Test-tube Respirometer. (a) glass plug, (b) rubber stopper, (c) test tube, (d) germinated peas, (e) water bath, (f) cardboard disk, (g) NaOH solution, (h) manometer, (i) mercury (j) rubber band, and (k) wooden strip.

cardboard disk, which should be coated with paraffin, is pushed down the test tube to a point about 2 cm. above the surface of the sodium hydroxide solution. At this time the apparatus is ready for the plant material. Garden peas sterilized with 0.4% Uspulun and germinated between moist blotting papers for 3 or 4 days have proved highly satisfactory for this work. Fifteen grams of this material is placed in the tube and the rubber stopper containing the manometer inserted in the mouth of the test tube. The respirometer *with the glass plug removed* may then be placed in the water bath maintained at the desired temperature. The respirometer is held in place by means of a rubber band stretched tightly between the manometer tube and one of several small wooden strips upon which the water bath rests. (Fig. 1). About 15 minutes with frequent stirrings of the water bath should be allowed for the temperature adjustment to take place.

After sufficient time has elapsed for the temperature adjustment, the glass plug should be inserted in the rubber stopper, the manometer tube tapped vigorously and the mercury levels in the manometer marked. This may be done by sticking a narrow strip of gummed paper on the back of the manometer tube, level with the meniscus of each of the two mercury columns. The time at which the manometer tube is tapped should be taken as zero time. Manometric readings may then be taken at stated intervals of time (5, 10 or 15 minutes) with the aid of a small millimeter scale. They must be preceded by a vigorous tapping of the manometer tube to facilitate the adjustment of the mercury columns.

When sufficient readings have been taken to satisfy the purpose of the experiment, the respirometer is removed from the water bath and the volume of the air space within the test tube determined. This is done by removing the glass plug and inserting the narrow outlet tube of a burette loosely through the hole in the rubber stopper. The volume of water required to fill the respirometer gives the volume of the internal atmosphere. The small gas volume in the capillary manometer tube may be neglected without involving serious error.

Data collected should include the manometric readings at known times, the volume of the air space within the loaded respirometer, the temperature of the water bath and the barometric pressure. In the absence of a barometer, an atmospheric pressure of 760 mm. may be assumed without seriously impairing the usefulness of the method.

Calculations of the amounts of oxygen absorbed are based upon the data just indicated and the gas laws respecting changes in volume with regard to pressure and temperature. Changes in the mass of the gas, due to the absorption of oxygen, are neglected for the sake of simplicity. While this factor should be considered for the higher accuracy required in research, it is believed that their inclusion here would only complicate matters for the student. The method of calculation may be seen from a study of the following table.

Respiration data for 15 gms. of germinated peas at 25°C.

TIME	Manometer Reading			Internal* Pressure	Internal Volume†		Oxygen‡ Absorbed
	Left Arm	Right Arm	Total		at 25°C.	at 0°C.	
0 hrs.	0.0 mm.	0.0 mm.	0.0 mm.	760. mm.	56.5 cc.	51.8 cc.	0.0 cc.
0.25 hrs.	4.5 mm.	4.5 mm.	9.0 mm.	751. mm.	55.8 cc.	51.1 cc.	0.7 cc.
0.50 hrs.	10.5 mm.	10.5 mm.	21.0 mm.	739. mm.	54.9 cc.	50.3 cc.	1.5 cc.
0.75 hrs.	15.5 mm.	15.5 mm.	31.0 mm.	729. mm.	54.2 cc.	49.6 cc.	2.2 cc.
1.00 hrs.	21.5 mm.	21.5 mm.	43.0 mm.	717. mm.	53.3 cc.	48.8 cc.	3.0 cc.
1.25 hrs.	28.0 mm.	28.5 mm.	56.0 mm.	704. mm.	52.3 cc.	47.9 cc.	3.9 cc.
1.50 hrs.	34.5 mm.	34.5 mm.	69.0 mm.	691. mm.	51.3 cc.	47.0 cc.	4.8 cc.

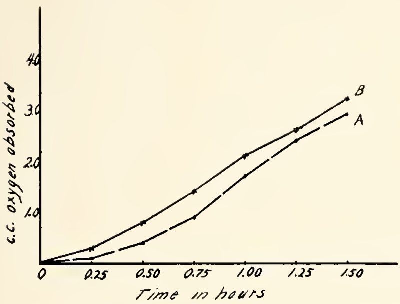

Fig. 2—Respiration of germinating peas at 25°C. Duplicate determinations for two 15 gm. (fresh weight) samples.

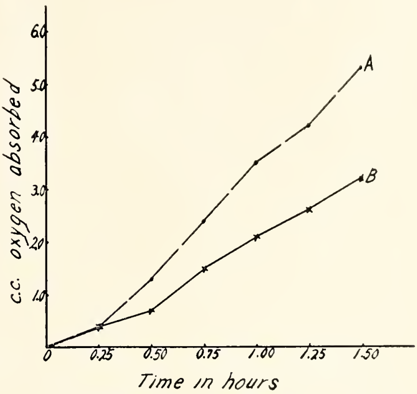

Fig. 3.—Effect of temperature on the respiration of germinating peas. A = oxygen absorption of 15 gms. of peas at 30°C. B = same at 20°C.

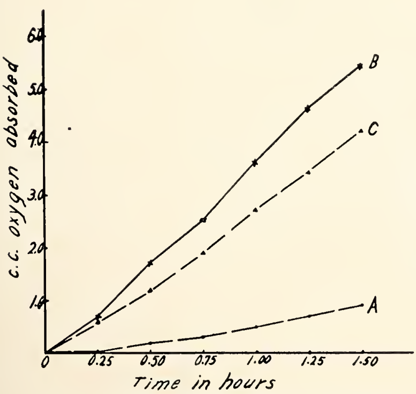

Fig. 4—Respiration of germinating peas at different stages of germination. A = 1 day from sterilization, B = 3 days and C = 5 days from sterilization. Temperature 25°C. Data taken on 15 gm. samples.

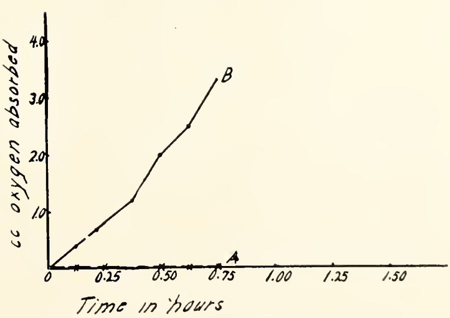

Fig. 5—Effect of steam treatment on respiration of germinating peas at 25°C. A = oxygen absorption of 15 gms. peas autoclaved for 10 minutes. B = same for untreated peas.

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*Internal atmospheric pressure = barometric pressure − manometer reading.

†Internal volume = measured air space volume $\times \dfrac{\text{Internal pressure}}{\text{Barometric pressure}}$

This value for the internal volume at 25°C. is then reduced to standard temperature by multiplying by the factor $\dfrac{273}{273 \text{ plus } 25}$ or 0.916.

‡Oxygen absorbed at N. T. P. = Initial internal volume − internal volume at any given time.

The course of aerobic respiration as determined by the absorption of oxygen may be shown graphically by plotting oxygen consumption against time. This has been done for duplicate determinations as shown in figure 2. Curve A is plotted from the data given in the preceding table. A second experiment performed in duplicate gave a very similar set of parallel curves.

In addition to demonstrating the normal course of respiration, experiments may also be performed by the student to demonstrate the relation of temperature to respiration. Figure 3 represents a typical set of duplicate experiments in which the respiration of two similar samples of germinated peas was determined. The temperatures studied were 20 and 30° C. In the graph the volumes of oxygen absorbed in each case are again reduced to normal temperature and pressure conditions. This makes the results entirely comparable.

That the respiration of germinating seeds changes with time may be demonstrated experimentally as is shown in figure 4. These results, based upon equal fresh weights of material, indicate that the magnitude of respiration increases with time from sterilization for a period of several days and then decreases markedly.

Figure 5 shows the result of killing the fresh material with steam. The peas autoclaved for 5 minutes showed practically no oxygen absorption while the control experiment exhibited the usual rate of respiration.

Other factors influencing respiratory activity, such as the action of certain cell toxins, may occur to the student and be tested quantitatively with this apparatus. Once the student's interest is aroused, he will probably wish to try various experiments for himself.