A New Method for the Quantitative Measurement of Gases

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The reversible respiratory-photosynthetic equation $C_6H_{12}0_6 + 60_2 \leftrightarrows 6CO_2 + 6H_2O + 647$ cal. is probably the basis of more discussion in general courses in botany and plant physiology than any other fundamental process; yet, in spite of its recognized importance, less individual laboratory work is based upon it than upon most any other basic activity. This is due chiefly to the fact that the apparatus necessary to demonstrate quantitative measurements is not adaptable to individual student use in large classes, and consequently, the process remains very much of an abstraction in spite of a possible lecture table demonstration.

To show the possibilities in the equation it may be well to take it up after the manner in which we have used it at Illinois State Normal University in large freshman sections, it being understood, of course, that no originality is claimed for anything except the method of measuring the interchange of gases. Barley, or any other desirable seed, is germinated until a well developed plumule is formed. This germination demonstrates the necessity of oxygen, water, imbibition, osmosis, and proper temperature. It is then shown that digestion of stored carbohydrates has taken place through enzyme action by means of the well known iodine and Fehlings' Solution tests. The use of the digested products, dextrose, and oxygen according to the respiratory equation is then demonstrated quantitatively by means of an exceedingly simple respirometer as follows: a dozen seedlings are placed in the upper closed end of a fermentation tube where they are readily held by their spider-like roots. The seedlings are separated from the outside air by pouring enough water into the bulb end of the fermentation tube to form a trap. The seedlings are thus enclosed in an atmospheric "universe" of their own and results are measured the following day or after any other desired interval.

Before the measurements are made, the seedlings are shaken down into the water trap and removed with an ordinary curved forceps. After the height of the air column in the closed end of the tube has been measured, the bulb is filled to overflowing with a 20 per cent sodium hydroxide solution. The thumb is placed over the mouth of the fermentation tube and the air of the closed arm and the solution of the bulb are thoroughly shaken together for a few minutes to absorb the CO_2 present. The tube is then tilted to run all the solution back into the bulb leaving all the air in the closed arm. The thumb is then carefully slipped from the mouth of the fermentation tube and the subsequent decrease in the length of the air column is measured. The difference between this and the first reading gives the amount of CO_2 given off as measured by the amount of CO_2 absorbed by the sodium hydroxide solution. This amount is then calculated on a percentage basis. The amount of O_2 left is then determined in the closed arm. This is done by removing with a medicine dropper about

half of the sodium hydroxide solution from the bulb of the fermentation tube and replacing it with an equal quantity of a 7 per cent pyrogallol solution. The thumb is again placed over the mouth of the fermentation tube and the operation repeated as in the determination of CO_2. The thumb is removed, the height of the air column measured and this reading (the third) subtracted from the second reading gives the amount of O_2 left in the tube as measured by the amount of O_2 absorbed by the sodium-pyrogallol solution. This difference, calculated upon reading number one, gives the percentage of O_2 left by the seedlings.

In the use of the above data it is to be remembered that air contains approximately 20.8 per cent of oxygen. If the test as run showed approximately 20.8 per cent CO_2 and no oxygen, then it may be seen that the amount of CO_2 formed is the equal of the O_2 consumed as demanded by the equation. If the test showed 10 per cent CO_2, then the amount of O_2 left should have been 10.8 per cent as required by the equation (i. e. the CO_2 formed should equal the O_2 consumed). Such results will be obtained by starchy seeds, for seeds rich in oil and protein give a different respiratory quotient and hence the amount of CO_2 used will not be the equal of the amount of O_2 consumed. Likewise, if an experiment is run too long, anaerobic respiration may upset expected results.

The calories liberated in the equation may be demonstrated by the well known method of inserting a thermometer into a plugged Thermos Bottle filled with germinating seedlings. That respiration is a chemical oxidation dependent upon an oxidizing catalyst or enzyme may be shown by testing cut seedlings for peroxidase with an alcoholic solution of gum guaiac containing some hydrogen peroxide.

In the extension of the equation to photosynthesis, barberry twigs are forced up into the closed arm of each of two fermentation tubes with the cut ends resting in the water traps. The tubes are set up at any time during the day. One tube is kept in the dark until the following morning while the other tube is placed in a window in order to get the direct rays of the sun early in the morning. At nine o'clock in the morning, or later, the twigs are carefully removed with a forceps. Each tube is then tested according to the method previously outlined. It will be found that the CO_2 found in the closed arm of the tube in the dark exactly equals the O_2 that was originally in the air, while the amount of O_2 found in the tube in the light equals 20.8 per cent thus proving that all the CO_2 formed over night (20.8 per cent) has been replaced by O_2 as demanded by the photosynthetic equation.

If the experiment is not run long enough, partial results are obtained which may be explained as in the case of the barley seedlings.