CELL RESPIRATION STUDIES.

I. A Microspirometer for the Continuous Study of the Oxygen Absorption by Living Cells.*

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The study of the ultimate metabolism of living organisms resolves itself into the analysis of the process in individual cells. While certain facts may be learned from the study of unicellular organisms, the specialized cells of the more complicated structures of mammals present additional problems. For the study of the respiration of such cells, numerous methods have been devised. Some depend on the oxidation or reduction of chemical agents, and others depend on the principle of gas analysis. The criticisms of most of the published methods are that the cells are exposed to abnormal conditions of environment, temperature, and osmotic pressure, and that the quantitative methods do not permit the continuous study of the same cells over long periods of time. It is possible to note the disappearance of oxygen in whole blood by determining the oxygen content at the beginning and at the end of a definite period, but in the actual chemical analysis the cells are killed. In such methods the number of observations is limited and the composition and identity of the cells are naturally not homogeneous.

To obviate these criticisms, an apparatus was designed to allow quantitative measurements of the volume of oxygen over a sample of cells. In the experiments with this apparatus seeds, pieces of tissue, and blood corpuscles were used, without disturbing their vital processes. The living structures during the time of the experiment

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were kept at a uniform temperature and in intimate relation with the respiratory medium.

The principle on which this microspirometer works is similar to that of most forms of closed space respiration apparatus. It has the advantage of this type of apparatus in that very accurate determination of the oxygen consumption can be obtained with comparative ease over short periods of time. It may be used for experiments involving a composition of gases differing from the atmosphere. The variations in the volume of the oxygen in the respiratory chamber may be measured during a given interval of time if all the carbon dioxide produced is absorbed by a solution of potassium hydroxide. The difference in the volume at the beginning and at the end of a given period, when the temperature and pressure are kept constant, is a measure of the amount of oxygen absorbed. Readings of the volume of oxygen used may be made at regular intervals during the course of the experiment.

Among the several forms of closed space microspirometers described by others, the differential microspirometer for the direct measurement of the consumption of oxygen designed by Barcroft and Roberts (1) and later modified by Krogh (2), and by Warburg (3), and also the one used by Winterstein (4), present certain technical problems, when blood is used, which make the results difficult to interpret. The apparatus designed by Koehler (5), though easy to manipulate, is not practical for small amounts of blood. Various forms of apparatus such as the Van Slyke (6) and the Haldane (7) measure changes in oxygen content, but the cells are killed in the process.

Such factors as the difference in vapor pressure between the material examined and the absorbing fluid, the difference in vapor tensions over the leveling fluids in the differential capillary tubes, the incomplete absorption of carbon dioxide, insufficient shaking or stirring, and the like, are sources of error which modify appreciably the results. The apparatus designed in this laboratory appears to eliminate these sources of error.

The Apparatus.

A diagram of the microspirometer described below is shown in Fig. 1. It is constructed of Pyrex glass.

For convenience the whole apparatus is mounted on a board and placed on a stand so that it can be put in a water bath with the level of the water just above the transverse connections and stop-cocks.

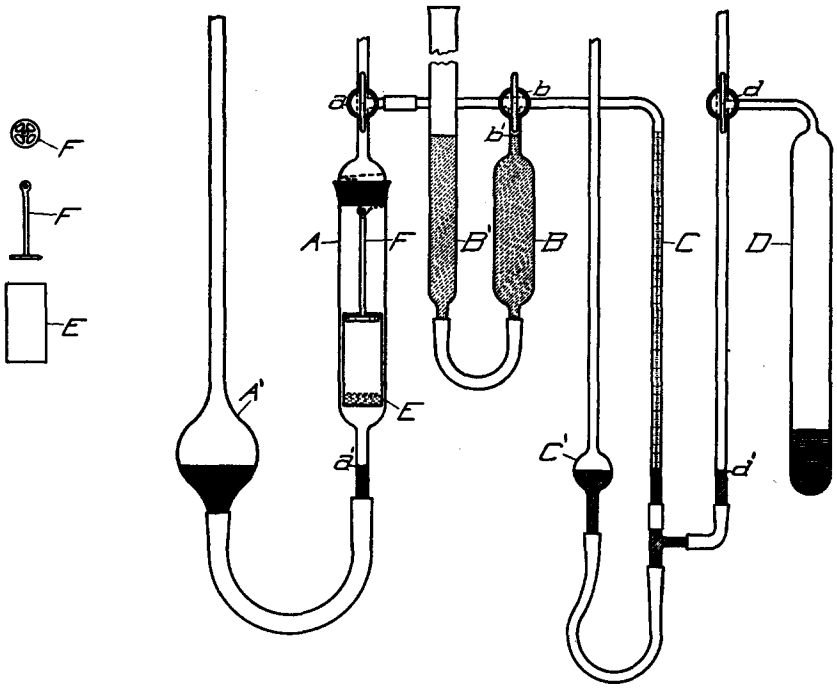

FIG. 1. A, metabolism chamber with reservoir A' for mercury. B, absorption chamber with reservoir B' for potassium hydroxide. C, manometer tube with reservoir C' for bile solution. D, compensation tube. E, cup to hold material for study. F, stirring rod. At the left the face view shows the perforated disk. a, b, and d represent the three-way stop-cocks which are attached to the chambers with corresponding letters. a', b', and d' represent the level to which the solutions are raised when readings are taken.

The microspirometer consists essentially of four parts:

1. The respiration or metabolism chamber A which holds a small cup E into which the material to be studied is placed.

The outside measurement of the metabolism chamber A is 19 × 3.9 cm. The bottom narrows down leading through a capillary tube into a rubber hose connected with the mercury reservoir A', the diameter of which is 7 cm. The top of

the metabolism chamber is closed with a tightly fitting ground glass stopper, on which there is a three-way stop-cock leading from this chamber to the outside and to the absorption chamber. Inside the stopper there may be placed a small brass spring to suspend the stirring rod *F*. The stirring rod consists of a perforated glass disk, 2.3 cm. in diameter, on the end of a glass rod 6 cm. long. The cup *E* in the metabolism chamber is 6.5 cm. deep and 3.3 cm. in diameter.

2. The absorption chamber *B* which contains the potassium hydroxide solution to absorb the carbon dioxide in the air driven over from the metabolism chamber.

The absorption chamber measures 15 × 3.5 cm. Its outlet to the reservoir *B'* is 0.5 cm. in diameter and the reservoir *B'* is 45 × 25 cm.

The concentration of the potassium hydroxide used is 0.96 N.

3. The manometer tube *C* which indicates the change of volume resulting from the consumption of oxygen by the material studied.

The manometer tube has a bore of about 0.3 cm. and it is 30 cm. long. It is graduated into tenths of a cm. and has a volume of 2.2 cc. Calibration shows each cm. to equal 0.0735 cc. at 37.5°C. The reservoir *C'* is 3.5 cm. in diameter.

A solution of bile salt containing 23 gm. sodium chloride and 5 gm. sodium taurocholate in 500 cc. of water (8) is used in the manometer. This solution has the advantage of dissolving fat, and therefore runs evenly in the capillary tubes. It does not absorb oxygen or carbon dioxide to a significant extent.

4. The compensating tube *D*, which is attached to the manometer to correct for changes in temperature and barometric pressure after the apparatus is closed.

This chamber has the same volume (148 cc.) as the metabolism chamber *A*. For each cc. of material added to the latter an equal volume of water should be placed in the compensating chamber *D*.

Procedure.

The reservoirs attached to the apparatus are filled with the proper fluids. Enough mercury is placed in the reservoir *A'* attached to the metabolism chamber *A*, to raise the cup *E* to the top of the chamber when the reservoir is elevated. 2 or 3 drops of the bile solution are placed on top of the mercury in the metabolizing chamber *A* to prevent the mercury from adhering to the sides and thus interfering with the readings.

The potassium hydroxide solution is placed in the reservoir B' and allowed to fill the absorption chamber B to the mark b'. The manometer C and its reservoir C' are partly filled with the bile solution.

To make the apparatus absolutely air-tight the stop-cocks must be cleaned and lubricated with a suitable stop-cock grease (*e.g.* Ramsay) for each experiment.

When the apparatus is ready, the material to be studied is placed in the cup inside the metabolism chamber A. The ground glass stopper is well lubricated with stop-cock grease and sealed.

The entire apparatus is then placed in a water bath maintained at 37.5°C., or any other desired temperature. In the water bath used it was found possible to maintain a temperature of 37.5°C. with a maximum variation of 0.2°. The temperature may be observed every 5 to 15 minutes on a Beckmann metastatic thermometer, during the course of each experiment (1 to 5 hours). The water bath is heated with electric light bulbs, controlled by a mercury level thermostat. The water is kept in circulation by a motor-driven stirring apparatus. An hour is allowed for the apparatus to acquire the temperature of the bath. The microspirometer is left in the water bath throughout the experiment and readings may be made through a glass window in the side of the water bath.

When a fluid substance, as blood, is to be tested it may be added, after the apparatus is warmed, through a long, hollow needle, inserted through the stop-cock a.

Before observations are commenced all of the stop-cocks are opened to the air, then the mercury is leveled to the mark a', the potassium hydroxide is leveled to the mark b', and the bile solution is brought to the mark d' of the compensating tube. The manometer reading is noted and then the apparatus is closed to the air with the fluids at these levels. Stop-cock b is then closed so that the absorption chamber connects only with the metabolism chamber, which is closed off from the outside by stop-cock a. The compensating tube is closed so that it connects only with the manometer tube.

When blood or a fluid is studied it should be stirred constantly. To do so the mercury reservoir is raised and lowered slowly but continuously, so that the perforated disk suspended in the metabolism chamber agitates the fluid as it rises and falls. If agitation is unneces-

sary, as with solid substances, then at any desired period of time the mercury reservoir is raised and lowered several times to drive the gases over into the absorption chamber.

To make a reading of the new volume, the mercury is lowered slowly until the potassium hydroxide rises to the mark b'. When the solution is at this level, stop-cock b is turned so that the absorption chamber is closed off and the metabolism chamber is connected with the manometer. The mercury is now set at the mark a'. To make the reading, the bile solution must be brought to the mark d' in the compensating tube, and then the level in the manometer recorded. Leveling the bile solution at the mark d' corrects any changes in volume due to barometric and temperature variations. The diminution of volume recorded after this procedure is the result of the consumption of oxygen by the material studied, as the carbon dioxide produced has been absorbed. Any carbon dioxide remaining in solution does not influence the results as it is oxygen consumption that is desired. Any oxygen that may be in solution in the material studied and in the potassium hydroxide remains practically constant throughout the experiment, because the change in the tension is too small to affect measurably the amount of the dissolved gas. If a reading is taken before absorption of the carbon dioxide the quantity produced may be determined by reading the volume before stirring. This will give the difference between the oxygen consumed and the carbon dioxide produced, and after the absorption of carbon dioxide the remaining volume indicates the amount of oxygen consumed.

The apparatus must be calibrated by liberating or withdrawing known volumes of gas and measuring the change in level of the bile column in the manometer tube. The gas may be liberated chemically, or air or carbon dioxide may be added from a graduated pipette or syringe through stop-cock a. In experiments of this type the observed readings were what they should have been and well within the limits of experimental error. In the microspirometer described above, the volume change in the manometer tube multiplied by 3.2 gives the volume change in the metabolism chamber.

The error in reading the level of the bile solution in the manometer tube is 0.5 mm., which is equivalent to 0.00367 cc. of gas in the manometer or 0.01174 cc. in the volume of the metabolism chamber. The

bore of the capillary tubes, in which the mercury of the metabolizing chamber at a' and the potassium hydroxide at b' are leveled, is practically the same as that of the manometer, so that the error of reading these levels is no greater. When blood is placed in the microspirometer and the stop-cocks closed to the outer air, the gas in the chamber slowly increases in volume, during the course of 5 to 30 minutes. A decrease due to oxygen absorption by the cells becomes apparent after the maximum increase has been attained and equilibrium has been reached. The preliminary increase in volume may be the result of several factors. The blood cools slightly when drawn and there is an increase in the temperature when it is put into the apparatus. This may result in changed vapor tension as well as changed solubility of dissolved gases. It is also possible that, under the conditions of the experiment, methemoglobin may be formed, with liberation of oxygen. Spectroscopic examination of the blood after the observations had been completed were made by Dr. Mirsky and Dr. Anson, but the amount of methemoglobin, if present, was too small to be noted by the spectroscope.

The procedure used for studying blood has been as follows:

8 cc. of human blood are withdrawn from the median vein of the arm into a syringe containing a solution of heparin[1] as an anticoagulant. The syringe is gently agitated to thoroughly mix the heparin with the blood. If it is desired, the blood may be saturated with oxygen. 5 cc. are then put into the cup E of the warmed microspirometer, through a hollow needle, inserted in stop-cock a. The fluids are brought to a level at their respective marks and the apparatus closed to the outside air. Stirring is commenced at once and continued throughout the experiment in order to keep the blood oxygenated and continually in motion. Readings are taken at about 10 minute intervals. The rest of the blood may be used for quantitative morphological studies.

When isolated corpuscles are used instead of whole blood, the procedure is the same. The blood is drawn into a syringe containing heparin and then centrifuged. The plasma and white cells are removed separately. Enough of the plasma is then added to the cells to make up the desired volume. Quantitative studies of the cells in the resulting suspension may be made.

[1] 0.05 cc. of a 2 per cent solution of heparin is used for 5 cc. of normal blood. Twice as much is used when working with leucemic blood, with a high white blood cell count.

One may readily observe the consumption of oxygen by many forms of living material. At the suggestion of Dr. Alfred C. Redfield, sprouting radish and lupin seeds were used for this purpose and the rate of oxygen consumption was measured at frequent intervals over a period of 2 days. The results show a uniform rate of oxygen consumption with the growth of the seeds.

The amount of oxygen used by normal or tumor tissues is readily measurable by this spirometer.

In working with an apparatus of this type it should be borne in mind that the actual rate is a function of the shape and size of the vessel in which the material is exposed and the rate at which it is stirred. If the process which liberates or consumes gas proceeds at a uniform rate, and the rate of stirring (and, therefore, the rate of exposure of carbon dioxide, when formed, to the potassium hydroxide) is uniform, the rate per hour is the same at different times. If, under these conditions, the rate per hour differs from time to time, it is suggestive that the process does not proceed at a uniform speed. Data as to rate are comparable only when the stirring is uniform. The "lag" of the mercury in the capillary tube acts as a governor, controlling the speed of the stirring, and this helps to keep it uniform.

Comparative studies of the oxygen consumption of normal and pathological bloods are of value if they are carried out under similar technical conditions.

CONCLUSION.

A description is given of a closed space respiration apparatus which can be used to determine the amount of gas used or liberated by living blood or tissue cells, or chemical substances. Continuous observations can be made and repeated measurements recorded without interrupting the vital processes or destroying the cells.

Studies of the oxygen consumption by whole blood in normal individuals and in patients with leucocytosis and myelogenous leucemia, as well as by white cells suspended in plasma, will be reported in subsequent papers.

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