should be right side up that they may be read without the necessity of removing the specimen from the tray or case. In no instance should flies be gummed or mounted in any manner on cards, which are certain to obscure important characters.

Revision of other recommendations which occur in the preface should be made. Fine-mesh bobbinet is the proper material for nets; and white is the preferable color, facility of locating the fly in the net after capture outweighing any element of alarm to the fly prior to capture. In fact, the white net is very attractive to many flies, rare species often alighting thereon voluntarily in the field. As to size, the 22-inch diameter bamboo ring set in an unjointed three-foot light wooden handle is the most effective, specimens rarely escaping it even if the cast is made during flight. This is the net used by the veteran English field-naturalist, Mr. A. E. Pratt, in South America and New Guinea. It is sufficiently light to be easily wielded in one hand, and performs exceptional service.

The fly is best transferred directly from the net to the cyanide vial. The latter should be the 25 x 100 mm. flat-bottom clear-white shell vial, the cyanide enclosed in a wad of tissue paper and tightly wedged into the bottom, shredded tissue paper being placed loosely in the vial to prevent undue rubbing and contact of specimens, and closed with a soft cork stopper. Large and small flies should go in separate vials; such forms as bombyliids with pile that is easily detached must be kept separate, as well as culicids and other forms that might be injured by stouter flies or that might mess others with their scales, pile, exudations, or pollen. The judgment of the collector must guide him, and he should carry a liberal supply of the vials. The specimens may be left all day in such vials without injury, but should be pinned the same evening or at latest next morning. In dry climates they will not last well over night.

In giving measurements of flies, the length of one wing, and not the expanse, should be stated. The expanse is not a stable quantity, due to drying and faulty spreading; moreover, the wings of study material should not be spread.

As to the classification adopted, it is especially important to present a correct system in a work intended for beginners. Most systematists will criticize the inclusion of the fleas with the Diptera. The superfamily Muscoidea is made to include the entire calyptrate and acalyptrate divisions. The superfamily name *Cypseloidea* should be applied to the acalyptrate groups, while *Muscoidea* should be restricted to the higher calyptrates. The Muscoidea of the author are stated to produce ova as a rule, but there are very extensive groups of the higher calyptrates that deposit larvæ; in fact, the larvipositing species of calyptrates will probably easily exceed in number the ovipositing species. The Nematocera has recently been shown by Knab and others to be an unnatural group. In the pages of half-tone reproductions, the Cyclorrhapha are divided into Proboscidea and Eproboscidea, the latter comprising the Pupipara as opposed to all the other Cyclorrhapha; an unnatural arrangement, since the main Pupipara show close affinity with the Cypseloidea and not with the Syrphoidea. The Phoridæ are wrongly included in the acalyptrate series. The Bombyliidæ, and not the Braulidæ, are commonly termed "bee-flies."

With these few friendly criticisms, the book is commended as a very useful means of presenting objective instruction in dipterology.

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SPECIAL ARTICLES

A SIMPLE AND RAPID METHOD OF STUDYING RESPIRATION BY THE DETECTION OF EXCEEDINGLY MINUTE QUANTITIES OF CARBON DIOXIDE

IN order to arrive at a satisfactory knowledge of life-processes, it is necessary to have accurate quantitative methods by which the measurement of these activities can be made. One of the best means of accomplishing this is found in the study of respiration. The production of CO_2 is regarded[1] as the only reli-

[1] *Cf.* Tashiro, S., *Amer. Jour. of Physiology*, 32: 107.

able universal expression of respiratory activity in anaerobic and aerobic tissues in normal condition.

It is extremely important to possess a method of detecting very small quantities of CO_2 as it is given off by the organism in the normal environment. The excellent methods devised by Tashiro[2] for the detection of very minute quantities of CO_2 are unfortunately limited to the study of tissues which are not bathed by solutions. But many of the most important studies on respiration require that the tissues shall be immersed in solutions in order to measure the effect of dissolved substances on respiration. Moreover the methods of Tashiro do not enable us to determine the quantities of CO_2 produced from moment to moment as the reaction goes on and thus to construct the time curve, which is, in most cases, of primary importance.

These difficulties are overcome by the method here described. The method consists in adding an indicator to the solution containing the tissue and observing its color changes.

The indicator should possess the following qualities: (1) it should be non-toxic to the material; (2) it should not rapidly penetrate the tissues; (3) it should be sensitive to very slight increases in the hydrogen ion concentration due to CO_2; (4) it should have a suitable working range.

Phenolsulphone-phthalein with a range of color changes from PH^+ 6.5 to PH^+ 8.5 but with extremely sharp differentiations in color between PH^+ 7.0 and PH^+ 7.5, has been found to be very satisfactory.[3] Other indicators of various ranges of color change, such as phenolphthalein, alizarin sodium sulphonate, etc. (sulphonic acid salts being not readily absorbed by cells), are being studied as to their usefulness for such work.

When salts occur in the solutions used, the salt error for the indicator should be taken into account. Some indicators can not be used with certain salts on account of being precipitated out of solution, but experimentation alone can tell which, in the large list of accurately described indicators,[4] are best adapted to a particular need.

If the material is of the nature of seeds, algæ, or aquatic animals, the whole of which can be submerged, the following procedure is followed: A tube of non-soluble or Pyrex glass of the desired diameter and length (for small seeds, algæ, etc., 16 mm. diameter by about 4 to 5 cm. long is very satisfactory; tubes below 16 mm. diameter are not recommended) is closed at one end by fusion. A piece of rubber tubing about 7 cm. long is attached at the open end. It is best to boil the rubber tubing repeatedly previous to using it, in order to insure thorough cleanliness. The rubber tube, while attached to the glass tube, is dipped a few seconds into hot paraffin so as to put a thin coat on both sides of the rubber. The best grade of paraffin (58°–62° C. melting point) is used, and serves to prevent the rubber from possibly giving off substances to the solution and also is advantageous in giving a seal against the CO_2 of the air. Ordinary soft glass tubing (which gives off alkali) or parawax (which gives off acid) is not suited for accurate work. Pyrex tubes, in the absence of Jena glass, can be used to advantage, especially because all sizes can be obtained.

The material to be studied is placed in the glass tube with a definite number of c.c. of solution containing a definite number of drops of an indicator of known strength. The volume of solution used is always made as small as possible, consistent with the requirements for colorimetric work, but however small the volume of solution used, slightly more than enough to fill the glass tube must be taken. The paraffined rubber tube is then closed with two strong pinchcocks so as to exclude all air from contact with the solution. The paraffin on the rubber tube is prevented from becoming brittle before it is clamped, by working rapidly or if necessary by the use of a lukewarm water bath. In this case the CO_2 in the solution is

[2] Tashiro, S., *Amer. Jour. of Physiology*, 32: 137; *Jour. Biolog. Chem.*, 1914, p. 485.

[3] Lubs, H. A., and Clark, W. M., *Jour. Wash. Acad. Sci.*, Vol. V., No. 18, November 4, 1915.

[4] Höber, "Physik. Chem. der Zelle und der Gewebe," 1914, p. 171.

in equilibrium with the CO_2 of the air before the tube is clamped. The closed tube is inverted several times and the color of the solution is compared with a series of buffer solutions of known hydrogen ion concentration and the acidity at the beginning of the experiment is recorded. The tube can be put on a shaker, should conditions require it, and after any interval whatsoever, the tube is inverted a few times in order to stir the liquid and to get a uniform color throughout the solution and then by comparing it with the buffer solutions, the increase in hydrogen ion concentration is noted. This can be repeated any number of times and at any interval of time. Changes in the hydrogen ion concentration as small as from 2×10^{-6} to 1×10^{-6} can be detected in this way.

Much smaller differences in the hydrogen ion concentration of a solution can be detected by using distilled water nearly or entirely free from CO_2, or by using solutions in which the hydrogen ion concentration is low. The procedure when pure distilled water is used is the same as that just given except that while the tube is still in the bath ready for clamping, a CO_2-free gas is bubbled through the solution until, by comparison with the buffer solution, it is known that the solution in the tube is between PH^+ 7.0 and PH^+ 8.0. The tube is then clamped off as before and the hydrogen ion concentration is read at intervals by comparison with buffer solutions. If the solutions, due to added reagents, are quite acid, then the smallest amount of CO_2 that can be detected is increased. However it is often possible to add the same amount of alkali to each tube so as to decrease the hydrogen ion concentration at the start and in this event the method can become extremely sensitive so as to detect minute traces of CO_2. This is also true of many solutions in which the hydrogen ion concentration is very small.

When the respiration of roots is studied, the glass tube has both ends open and tubing on each end. The roots are inserted into one (very short) paraffined rubber tube, and by means of a pinchcock, the tube is clamped so that only a small space is left about the stalk as it protrudes. A low melting mixture is used to make the final seal about the plant. After the plant has been inserted, the paraffined tube is attached at the other end. The solution is then run in and the CO_2 expelled by bubbling hydrogen through. The paraffin, before clamping takes place, should be rather soft and pliable, and should it tend to become brittle it can be kept soft by being kept inside of a tube open at both ends and which is kept warm by a surrounding water bath. After clamping, readings are made as usual.

When the liquid used is pure distilled water, and is quite free from CO_2, a change in the hydrogen ion concentration as small as from 2×10^{-8} to 3×10^{-8} can be noted. The smaller the hydrogen ion concentration of the solution at the start of the experiment, the more minute the differences which can be detected. If the experiment is started with the solution in equilibrium with the CO_2 of the air, it is possible to ascertain whether or not the increased acidity has been due to the giving off of CO_2 or to acid excretions other than CO_2, by pouring the solution into another tube and (after shaking without the material) letting the solution come again into equilibrium with the air, and noting whether or not the solution returns to its original hydrogen ion concentration. Furthermore, by bubbling a CO_2-free gas through the solution at the end of the experiment and through a sample of the original solution, it is possible to find out whether acids other than carbonic acid have been given off. If at the end of an experiment it is found that acids other than carbonic acid have been given off, or that an unequal absorption of ions has taken place, so as to produce acidity, then the increase in the hydrogen ion concentration due to CO_2 can be obtained by subtraction. As it is important to know whether acids other than carbonic are given off by plant and animal tissues, experiments have been conducted upon the excretion of acids by plant tissue, the results of which will appear at a later time.

When it is desirable not to have the indicator in the solution during the experiment,

the method can be modified as follows. One end of the glass tube has a paraffined plug having two holes, while the other end has the usual paraffined rubber tube. One hole can be sealed shut if no stem is to protrude, while in the other hole a small glass tube containing the required number of drops of indicator is inserted with a solid glass plunger of equal diameter adjoining, and protruding from the plug. At the end of a given time the indicator is pushed into the solution by means of the airtight plunger and the reading is made rapidly. In such a modification, control tubes must be depended upon to give the hydrogen ion concentration of the solution at the start of the experiment, and, moreover, only one reading can be made from a single tube.

Pure block tin collapsible tubes have been found to be very useful but are very difficult to seal as compared with the paraffined rubber which is easily sealed. Experiments with seeds were run for an hour without any change in the control, and even though it may be possible to run experiments a much longer period without change in the control, yet it appears advisable to cut down the time of an experiment whenever possible; this the new method permits.

In making up buffer solutions,[5] the writer has found it advisable to recrystallize chemically pure salts several times, and whenever possible it is best to check up the accuracy of the buffer solutions with the aid of the hydrogen electrode.

The writer has found that a constant source of light such as has recently been described in Science[6] is almost indispensable for this work.

By using seeds with the coats removed and a relatively small amount of solution a color change can easily be detected within five minutes.

By this method we can compare the respiration of organisms in different solutions with great accuracy without knowing the actual amounts of CO_2 given off. We need only to compare the times required to produce the same change of color in the solutions. If we use a substance in solution which affects the change of color in the indicator, this substance must be added to the set of buffer solutions. If, for example, we are studying the effect of NaCl on the respiration of roots we put one lot of roots into a solution of NaCl and another lot into distilled water. We then prepare a set of buffer solutions to which we add NaCl so as to make its concentration the same as in the solution containing the roots. We add the same amount of indicator to the solution containing the roots and to the buffer solutions, and the changes of color are then comparable. We proceed in the same way with the distilled water or with any other solutions employed.

If we wish to know the actual amounts of CO_2 given off we may calibrate the indicator by a very simple method, as yet unpublished, due to Henderson and Cohn. We may then use an indicator which passes through a well-defined series of color changes as the amount of CO_2 increases. By observing these changes we can plot the amount of CO_2 against time. The resulting curve enables us to study the dynamics of the reaction and this is of primary importance for an understanding of the processes involved in metabolism.

[5] Michaelis, L., "Die Wasserstoffionen-Konzentration."

[6] Science, N. S., 42: 764, 1915.

SUMMARY

1. Respiration may be accurately followed by observing changes in the color of indicators added to solutions which contain organisms.

2. Exceedingly small amounts of CO_2 may be determined in this way with great accuracy.

3. As changes in color often occur in five minutes, the experiments may be shortened so as to exclude pathological changes in the organisms.

4. The simplicity of the apparatus makes it possible to carry on a large number of experiments at the same time.

5. The amounts of CO_2 produced in successive intervals can be determined without disturbing the organism. This enables us to study the dynamics of the process.

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