# AN INDICATOR METHOD OF MEASURING THE CONSUMPTION OF OXYGEN.

By W. J. V. OSTERHOUT.

(*From the Laboratory of Plant Physiology, Harvard University, Cambridge.*)

(Received for publication, September 11, 1918.)

The study of respiration has been greatly facilitated by using indicators to measure the production of carbon dioxide. It is desirable to employ similar methods for estimating the amount of oxygen consumed in respiration. With this in view the writer has investigated a number of substances, some of which appear to give promising results. The purpose of the present note is to describe very briefly the use of *Limulus* blood for this purpose.

The blood of *Limulus* (and of a number of other invertebrates) when shaken up with air absorbs oxygen and turns blue, but on standing is reduced and in consequence becomes decolorized. It has been pointed out by Alsberg[1] and by Harvey[2] that the decolorization is due (in large part at least) to the activity of microorganisms. It seemed to the writer that this might be utilized to measure the rate of consumption of oxygen in respiration.

The procedure adopted is as follows: Large horseshoe crabs are taken and an incision[3] is made at the joint in the middle of the body; the body is then repeatedly bent back and forth to expel the blood. After the blood has stood for a short time a clot forms from which the clear blue serum may be poured off. To a portion of this sufficient ether is added to form a saturated solution; this is allowed to stand in a stoppered bottle with occasional gentle shaking, until the ether is dissolved. A number of glass tubes are prepared by sealing

[1] Alsberg, C. L., *J. Biol. Chem.*, 1915, xxiii, 495. Alsberg, C. L., and Clark, E. D., *ibid.*, 1910–11, viii, 1. Alsberg, C. L., and Clark, W. M., *ibid.*, 1914, xix, 503. Alsberg, *ibid.*, 77.

[2] Harvey, E. N., *J. Gen. Physiol.*, 1918, i, 133.

[3] This enters the heart cavity. It is desirable to cleanse and dry the surface around the joint before making the incision.

one end and fitting to the other a piece of rubber tubing which can be clamped shut. Various dilutions of the blood are made (with a saturated solution of ether in sea water), each of which is placed in a separate tube. In one tube undiluted blood is placed. All the tubes are clamped shut to prevent the evaporation of ether.

The ether prevents the decolorization of the blood and the various dilutions furnish shades of color which serve as standards in subsequent experimentation.

If fresh water organisms are used in the experiments the procedure must be modified. Since the salt content of the blood is approximately that of sea water it must be reduced by dilution or by dialysis. If this is carried beyond a certain point precipitation occurs and the color of the aerated blood is paler in consequence.

The organisms are placed in glass tubes, provided with rubber tubing as described above, and blood (diluted or undiluted) is added; the tubes are then clamped shut, taking care to exclude bubbles of air. The observer then notes the time required to produce a definite change[4] in color (as determined by comparison with the tubes containing blood to which ether has been added).[5] It is desirable to observe the tubes against a dark background, preferably while facing the source of light. The best results are usually obtained by viewing the tubes from the end. For this purpose the tubes are inverted so that the rubber tubing is below; the organisms sink into it and the rubber tubing may be temporarily pinched off above the organisms so they cannot be seen.

As soon as a definite change in color is observed the tubes containing the organisms may be opened and shaken with air so as to restore the original color. There must be a control tube containing blood without organisms or ether; if this control becomes decolorized the experiment must be rejected, unless the decolorization is so slow as not to interfere with the result. Freshly drawn blood does not decolorize except very slowly; this is also true of freshly dialyzed blood, and of blood heated[6] for 5 minutes to 60°C. Blood preserved

[4] The tubes should be shaken from time to time.

[5] A "Daylight" lamp was used in most of the experiments.

[6] Heating at lower temperatures (40–55°C.) for a longer time may be preferable.

by the addition of ether may be freed from ether by a current of air[7] and does not decolorize rapidly until it has stood for some time. Blood which has been carefully dried to a jelly-like mass may be kept in this condition; it dissolves readily on the addition of water. But if dried to a hard, brittle mass it does not readily dissolve.

Under favorable conditions blood containing certain organisms,[8] (such as bacteria, or young *Limulus* in the trilobite stage) is quickly decolorized (in some cases within 2 minutes) and the time required for decolorization is remarkably constant.[9]

When the normal rate of oxygen consumption has been established by repeated determinations, reagents may be added and changes in the rate may be determined. In this way the relative rate under the influence of the reagent may be ascertained without knowing the absolute amounts of oxygen consumed in either case.

A variety of other substances, such as indigo-carmine, methylene blue, malachite green, etc., are also reduced and it is possible that some of them may prove more useful than *Limulus* blood. Experiments on this subject are being continued.

## SUMMARY.

The blood of the horseshoe crab (*Limulus*) absorbs oxygen and turns blue when shaken in air. In the presence of certain organisms which consume oxygen it is quickly decolorized. By measuring the time required for the change of color the rate of consumption of oxygen may be determined.

[7] The air should be filtered or washed to remove bacteria before entering the blood.

[8] The salt content must be adjusted to the needs of the organism; it may be quickly ascertained by titration.

[9] The normal rate is sometimes irregular, due to factors not fully understood. In these cases the results are rejected. The disturbing factors require further study.