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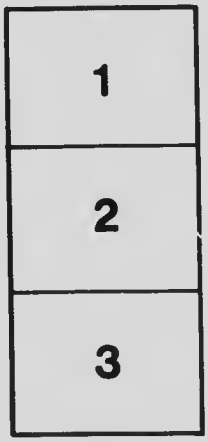

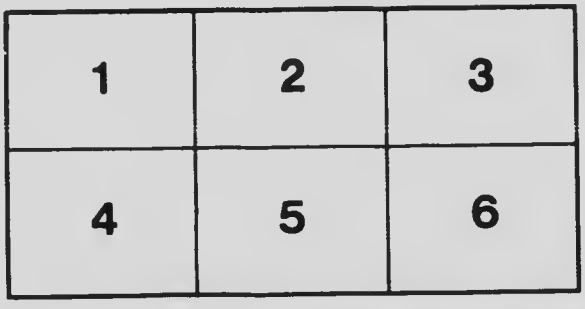

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No. 24: SIMPLIFIED GAS ANALYSIS, BY PROFESSOR J. J. R. MACLEOD and R. S. LANG

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SIMPLIFIED GAS ANALYSIS

No. IV. The Dissociation Curve for Oxygen and the CO_2 combining Power of Blood

BY

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SIMPLIFIED GAS ANALYSIS

NO. III. THE DISSOCIATION CURVE FOR OXYGEN AND THE CO COMBINING POWER OF BLOOD

By J. J. R. Macleod, M.B., and R. S. Lang, B.A., Toronto, Canada

For accurate determination of the relative amounts of reduced and oxyhemoglobin in blood exposed to atmospheres containing varying partial pressures of oxygen no method surpasses that of Barcroft and his coworkers.[1]

The principle of this method is to expose a small quantity of blood in a thin film on the walls of a relatively large cylindrical vessel (tonometer) containing a mixture of nitrogen and oxygen gases until equilibrium has become established between the partial pressure of the oxygen in the atmosphere and the absorption of oxygen by the blood. Some of the blood is then transferred to a bottle connected with a differential manometer and shaken with dilute ammonia water, by which it becomes laked and takes up its maximal load of oxygen, thereby causing shrinkage in the volume of air, the degree of which is indicated by the manometer. The oxygen-saturated blood is then shaken with ferricyanide of potassium which dislodges the oxygen and causes the pressure in the bottle to rise. From the displacement of the fluid in the manometer in the two observations, the percentage of saturation of the blood with oxygen is readily calculated.

For use by a class of students the method is not practical because of the difficulty of providing suitable mixtures of oxygen and nitrogen with which to fill the tonometer and because of the expense of the differential manometer. The first of these difficulties is overcome by exposing the blood to a partial vacuum instead of a mixture of gases, a principle which has also been applied by W. G. Macallum, who uses a modified Van Slyke pipette both as tonometer and analysis apparatus.[2] The advantage of a partial vacuum over a mixture of oxygen and nitrogen is that the partial pressure of oxygen is readily calculated from the degree to which the tonometer is evacuated, measured by a barometer. In the present method the blood after exposure to partial vacuum is transferred to a simple form of differential blood gas manometer in which there are no glass stopcocks, pressure adjustment being made by the use of the pressure adjuster described elsewhere.[3] Even with these simplifications, the technic is by no means easy, but the great importance of having the student clearly understand the principles of the method used for securing the data necessary to plot the dissociation curve amply repays the time he devotes to the experiment. Indeed without actually doing this experiment, it is the opinion of the writer that the average student rarely acquires any clear conception of the [illegible] tion of the blood. After he has conscientiously performed the [illegible] he other hand, the whole problem becomes clearer, even although

absolutely correct may not have been carried. A laboratory session of at least three continuous hours is essential, and the students can most profitably work in groups of three each.

DESCRIPTION OF THE APPARATUS

The Tonometer. This consists of a wide glass tube (the tonometer T, fig. 1) of fairly stout glass, tapering down to narrow tubes at both ends. The capacity should be at least 200 c.c.[1] The narrow tubes are connected with thick-walled (pressure) rubber tubing which should be wired on to the glass tubes. The rubber tubes are closed by screw clips (1 and 2). File marks are made

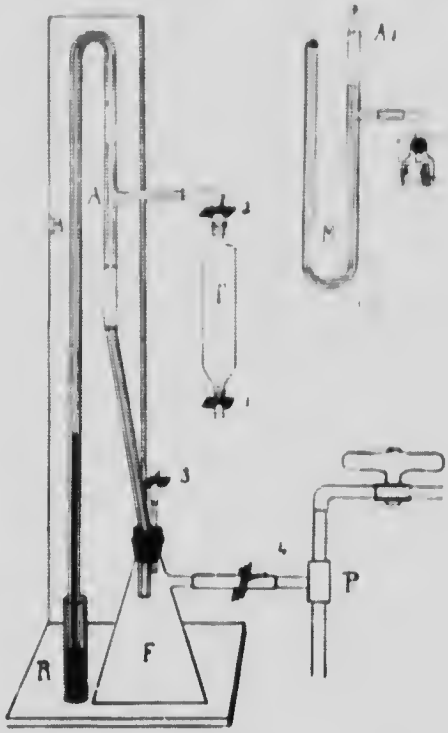

Fig. 1

one of the tapering ends of the tonometer, the distances between them corresponding approximately to one cubic centimeter.

The Barometer. This consists of a vertical, thick-walled glass tube about 1.25 meters long and of about 3 mm. bore bent on itself near one end, and with the other end dipping into mercury contained in a wider flat-bottomed (specimen) tube (the mercury reservoir) closed by a perforated cork. The barometer tube and reservoir are firmly mounted on a stand furnished with a millimeter scale which is attached to the stand in such a way that it can be adjusted to bring its zero to the surface of mercury in the reservoir, as this varies at different pressures. The free end of the barometer tube is connected by rubber pres-

[1] It would be preferable to use a tonometer two times as large since this would diminish error due to the addition of the gas given off from the blood.

sure tubing to a glass T piece (*c c*), one limb of which is similarly connected to a stout walled filtration flask (*F*) joined to a good water pump (*P*). A capillary tube closed by a piece of rubber tubing and a screw clip (*2*) also passes through the stopper of the flask.

The Differential Manometer and Gas Bottle. This apparatus requires a piece of narrow bored glass tubing (bore 1 mm.) bent into a U shape with one limb about 200 mm. long and the other about 150 mm. (*M* in illustration). To the shorter limb is connected a T piece of narrow bore tubing, which should be fused to it, although rubber pressure tubing is quite satisfactory. One limb of the T piece is connected with red rubber tubing of moderately thick wall, closed by a pressure adjuster (*Adj.*) as described elsewhere.* By pinching the rubber tubing lying over the lateral hole of the adjuster, the manometer is brought into communication with the outside so that the air in the bottle and manometer are brought to atmospheric pressure. The other tube of the T piece is connected by pressure rubber tubing with a glass tube which passes through a rubber stopper that accurately fits a small wide-mouthed bottle (*B*) of about 3.5 c.c. capacity. Finally a small flat bottomed test tube 18 mm. high and 6.7 mm. diameter is required, *I*. Instead of attaching the T piece to the short limb of the manometer, it may be inserted in the rubber stopper, one limb being connected with the manometer, which in this case simply consists of a U tube, and the other with the pressure adjuster. The fluid used in the manometer is olive oil.

TECHNIC OF ANALYSIS

The tonometer is rinsed out with physiologic saline and is connected with the side tube of the barometer T piece, *A*. The pump *P* is turned on with screw clips *1* and *3* closed, but screw clip *2* open and the pressure lowered until the mercury stands at a constant level in the barometer. Screw clip *1* is closed and the mercury observed to see whether there is any leak. Provided there is none, clip *3* is cautiously opened and the mercury allowed to fall almost to the level in the reservoir (*R*); clip *2* is tightened, the tonometer, *T*, removed, and the pump turned off. Defibrinated or oxalate blood (whipped ox blood is most suitable for large classes, but in any case blood from an etherized animal must not be used) is now sucked into the tonometer, by placing some of the blood in a small evaporating dish and cautiously loosening clip *1* with the rubber tube dipping into the blood; *3* to *4* c.c. of blood should be allowed to enter the tonometer. This is then reattached to the T piece *A* of the barometer and with clips *2* and *3* open (but *1* and *3* closed) the pump is turned on and the mercury allowed to rise as far as it will go, when clip *1* is closed and the pump turned off. Clip *3* is now cautiously opened until there is a partial pressure of about 20 mm. Hg oxygen in the tonometer.†

* This Journal, [illegible] 69.

† This is computed as follows: After suitable adjustment the standard barometer in the room is read and from the reading is subtracted the tension of aqueous vapor at the temperature of the room (for table see page 420 of this Journal, Vol. III). The difference gives the pressure in mm. Hg of an atmosphere of dry air. Since air contains 20.96 per cent oxygen, the partial pressure of this gas in the tonometer must be equal to $\frac{20.96}{100}$ of the difference between the height to which the mercury is raised in *B* and the corrected barometer reading. Thus, suppose the room barometer is 754.4 mm. and aq. tension 17.4 mm. the corrected barometer reading is 754.4 17.4 = 736 mm. Then $\frac{20.96}{100} \times 736 = 154.2$ mm. O_2.

20 mm. O_2 = $\frac{20 \times 736}{154.2}$ = 95.45 mm.

Hence the mercury in the barometer must be raised to 736 − 95.45 = 640.55 mm. above the level in the [illegible]

When the mercury has reached this level, or one near it, clip *3* is closed and the height at which the mercury stands very accurately noted. Clip *2* is then closed, after which the mercury is allowed to fall to zero by opening *3*. The tonometer is now removed and rotated so that the blood becomes spread out as a thin film on the walls, after which it is placed in a water bath kept at about 40° C. in which it is constantly rotated for about 15 minutes.

On removal from the bath the pressure in the tonometer must again be measured. For this purpose the tonometer is reattached to *A* and the pump is turned on (with *3* closed) until the mercury has risen to the level at which it previously stood. Clip *4* is closed and *2* opened. If there has been no leak, and time has been allowed for the tonometer to cool down, there will be practically no difference between the two readings. If a difference of more than 5 mm. is observed it must be noted and the pressure prevailing in the tonometer taken as the average between the two readings.

Meanwhile 3 c.c. of freshly prepared weak ammonia water containing a trace of saponin (0.5 c.c. aq. ammonia in 500 c.c. water) has been placed in the blood gas bottle *B*. A pointed glass tube about 30 mm. long is now attached to the rubber tubing of the tonometer and this is removed from the barometer and held in vertical position above the bottle. The screw clip *2* is opened so that the air enters the tonometer, the clip *1*, is then cautiously opened to let a drop or two of blood flow out from the tip of the glass tube,* and after closing it again the end of the tube is wiped free of blood and placed in the bottle so that it dips under the ammonia solution. Clip *1* is now cautiously opened and about 1 c.c. of blood allowed to flow under the ammonia water. If this is done carefully the blood does not mix with the ammonia water which floats on the top of it as a layer and so prevents any diffusion of oxygen between the blood and the air. The bottle is firmly closed by its stopper, the pressure adjuster being meanwhile held open so that the level of the clove oil in the manometer is not disturbed. The bottle must now be submerged in a water-bath containing water at about room temperature, in which it is left until, with the adjuster closed, no further contraction of volume, due to cooling, is observed to occur.

The bottle is now removed from the bath and vigorously shaken so that the blood becomes laked and absorbs O_2 from the atmosphere of the bottle. After replacing the bottle in the bath and allowing time for cooling the difference between the levels of clove oil in the two limbs of the manometer is noted. With the adjuster open to the outside the stopper is removed from the bottle and about 0.25 c.c. of a freshly prepared saturated solution of potassium ferricyanide is placed in the small flat-bottomed test tube which is then lowered by means of a forceps into the fluid in the bottle, without allowing any of the ferricyanide to mix with the laked blood. After reinserting the stopper and cooling, the bottle is again removed from the bath and shaken so that the ferricyanide by mixing with the laked blood drives off the loosely combined oxygen and raises the pressure, which is measured by the manometer.

The relative amounts of reduced and oxy-hemoglobin present in the blood are proportional to the first and second readings of the manometer; when all is reduced hemoglobin the diminished pressure (shrinkage) recorded in the first

*Enough blood should be run out to bring the meniscus of blood in the tonometer to the lower file mark

shaking of the bottle is practically the same as the increased pressure recorded in the second. They will not be exactly the same, since the volumes of bottle and tubing in the two cases are not the same, but the error thus incurred is inconsequential for most purposes (cf. Boyle's law).

The calculation of the percentage saturation of hemoglobin with oxygen is made by subtracting the first reading from the second, dividing by the second reading and multiplying by 100. Suppose in the observation made at 20 mm. partial pressure of O_2 the first reading is 24 mm. and the second, 108, then

$$\frac{108 - 24}{108} \cdot 100 = 77.7\%$$ HbO and 22.3% Hb.

The result must now be plotted on coordinate paper with the percentages of HbO along the ordinates and the partial pressures of oxygen on the abscissæ.

The observation is repeated at different pressures and by joining the points, the dissociation curve for blood is obtained. Care must be taken to see that the bottle is sufficiently shaken so that the partly reduced blood absorbs all the oxygen and gives it up again with ferricyanide. It is particularly in the latter operation that care must be taken.

The influence of carbon dioxide in lowering the dissociation curve can be readily shown by the method. The procedure is as follows: After the pressure has been reduced to the desired degree in the tonometer, the latter is placed in a horizontal position so that the blood lies along the walls, free of the ends. A CO_2 generating apparatus (Kipp's) or a bottle containing this gas is then connected by suitable tubing with the free end of the tonometer, care being taken before making the connection, to fill the tubing with CO_2. To accomplish this a slow stream of the gas is maintained and the air in the tubing beyond the screw clip (*1*) is squeezed out before connecting with the CO_2 generator. The most suitable partial pressure of CO_2 to work with is 40 mm. which is secured by cautiously opening screw clip *1* until with clip *2* open, but *3* and *4* closed, the mercury descends through 40 mm. in the barometer. Clips *1* and *2* are then tightly screwed down, and the tonometer removed, the further procedure being exactly as described above.

The effect of the 40 mm. of CO_2 will be found in the above example where a partial pressure of 20 mm. O_2 was used to reduce the percentage of HbO from 77 to about 35.

THE CO_2-COMBINING POWER OF THE ALKALINE RESERVE OF THE BLOOD

After completing the estimations necessary for finding the percentage of oxyhemoglobin, in the experiments in which CO_2 is present in the tonometer, it is of interest to determine the amount of this gas with which the blood has combined. This will represent its ability to act as a buffer towards foreign acids. To perform the estimation it is necessary, however, to measure accurately the amount of blood which is removed from the tonometer to the blood gas bottle. This can readily be done by attaching a 1 c.c. pipette to the tubing of the tonometer (beyond clip *1*), a few drops of blood being allowed to escape from the pipette before delivering under the ammonia solution in the bottle, and precautions being taken not to take any of the upper layers of blood that had been exposed to full

atmospheric pressure when the tonometer was opened. This is done by removing the pipette from the tonometer before all the blood has run out.

To dislodge the CO_2 from the blood, the stopper is removed with the usual precautions and about 0.25 c.c. of a saturated solution of tartaric acid placed in the small test tube. After closing and allowing for temperature changes, the acid is shaken with the mixture of blood and ferricyanide, and the CO_2 thereby evolved measured by multiplying the displacement of the fluid in the manometer by a figure (the constant of the apparatus) obtained by a preliminary experiment in which a known amount of a standard carbonate solution is similarly treated.

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