An Apparatus for Microdetermination of CO₂ in Plasma

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The apparatus to be described in this communication was contrived as a result of the necessity to determine the concentration of gases in small quantities of blood. Essentially it is based on Van Slyke's method (Van Slyke and Neill, 1924). A similar instrument has been described by Natelson (1951) which has a special piston arrangement for moving the mercury up and down.

In the apparatus to be described here the mercury is moved by lowering or raising the levelling bulb. It is very simple to construct in any laboratory with glass-blowing facilities. Although only about 0.06 to 0.12 ml. samples of plasma are used for the test, the accuracy of the instrument is comparable to its full-size prototype. The micro-apparatus is designed so that the manometric readings may be used directly in the tables and nomograms given by Van Slyke for the full-scale apparatus.

The Micro-apparatus

The instrument consists of a gas reaction chamber, a connecting Y-tube, a manometer, and a levelling bulb (mercury reservoir). The dimensions of the assembled parts are shown to scale in Fig. 1 and a photograph of the complete apparatus is presented in Fig. 2.

The materials required for the construction of the micro-apparatus are three stopcocks, pyrex capillary glass tubing blown with bulbs at predetermined places, and latex rubber vacuum tubing (4 mm. bore, outer diameter 10 mm.). The capillary tubing should be of uniform bore of 2.2 or 2.3 mm. (narrower capillaries cause bubbles of air to be trapped) for the extraction chamber assembly, and of 3 mm. bore for the manometer.

Gas Reaction Chamber.—This consists of the extraction chamber, a 5 to 6-ml. bulb (Fig. 1E) connected above it by means of a capillary glass tube 30 mm. long with a smaller bulb (D) of 0.5 ml. capacity. From here the capillary continues to the upper stopcock (A). This section (D–A) is about 90 mm. long and is calibrated in 0.01 ml. divisions; its total volume is 0.4 ml. The stopcock connects the chamber with a small narrow cup of 2 ml. capacity (L); through this cup, the fluid to be tested is introduced into the extraction chamber. Below the extraction chamber (E) a 10-mm. long capillary tube leads down to a large bulb (F) of 1 ml. capacity, and then 35 mm. further to a second smaller (0.5 ml.) bulb (G). The bulbs F and G prevent fluid entering directly into

FIG. 1.—Schematic diagram of the micro-Van Slyke apparatus (scale $\frac{1}{2}$). Alternative positions of levelling bulb (4) indicated by broken line: I=position 1; II=position 2.

the manometer when the levelling bulb is moved quickly; they also facilitate expulsion of air from the apparatus.

Y-Tube.—The Y-tube is connected tightly, glass-to-glass, by latex vacuum rubber tubing (4 mm. bore, outer diameter 10 mm.) to the reaction chamber at one end and to the manometer at the other. The third arm of the

FIG. 2.—Photograph of the micro-Van Slyke apparatus.

Y-tube leads through stopcock (B) to the levelling bulb (4).

Manometer.—The manometer consists of a uniform 3 mm. bore pyrex capillary tube graduated at 1 mm. intervals from 0 to 500 mm. If no facilities for glass marking are available, a plastic transparent ruler may be used; it is attached in front of the capillary. Below the zero point the capillary is blown into two bulbs (H and I) which are separated by a 10 mm. capillary tube. Bulb H is of 0.5 ml. volume and bulb I of 1 ml. Above the scale the manometer capillary tube is blown into a small bulb (K) which is connected through stopcock (C) to a small cup (M) which serves as a safety bulb against spilling the mercury.

Levelling Bulb.—The levelling bulb (mercury reservoir) has a volume of 25 ml. and is connected to the Y-tube

by means of a 100–120 cm. long latex vacuum tube.

When new latex rubber tubing is used, it is filled with a carbon tetrachloride solution of silicone (silicone fluid 200, Dow Corning Co.). After two minutes the tube is emptied and left wet overnight. The next day pieces of cotton wool are pushed through the rubber tubing with the aid of polyvinyl tubing until the cotton pieces leaving the tube are quite dry. This treatment results in coating the inner rubber surface with silicone and prevents the chemical reaction of rubber with mercury.

Stopcocks.—The stopcocks are equipped with a pressure lock which ensures an air-tight seal. An expanded view of the stopcock assembly is shown in Fig. 3. (Similar stopcock adapters are now available from E. Greiner Co., New York.)

In order to secure a tight fit (1) grease should be applied to the plug with the tip of the finger, (2) the plug is pushed in without turning until the entire surface of contact becomes clear and transparent, and (3) the pressure lock is attached. (For this purpose we recommend “apiezon” M grease supplied by W. Edwards and Co., London.)

Filling the Apparatus with Mercury

After assembling the apparatus, as shown in Fig. 2, the mercury is poured into the levelling bulb (4). By opening stopcock B the mercury is introduced into the reaction chamber and the manometric arm. This is done while the upper stopcocks A and C are open. Mercury is moved up and down several times by raising

FIG. 3.—Enlarged view of a stopcock with safety pressure lock.

and lowering the levelling bulb. Finally, when the mercury reaches the neck of the cups L and M, the stopcocks are closed. Then a vacuum is produced by lowering the mercury and thus releasing air bubbles from it. In order to check that all air had been extracted, the mercury is raised again to the stopcock and a metallic click should be heard.

Calibration of Gas Reaction Chamber

An Ostwald pipette with a stopcock or a capillary pipette with 0.1 ml. divisions is used. The apparatus is filled with mercury till above stopcock A, which is then closed. A few drops of coloured water (indigo, carmine, or Evans' blue solution in water containing some bile salt) are introduced into the cup. The mercury is carefully lowered with the stopcock open so that the meniscus of the mercury just touches the stopcock and the bore is filled with water. A mark is made on the

narrow neck of the cup with glass ink and all water sucked off above the mark, then 0.1 ml. coloured water (Fig. 2) is introduced and allowed to run into the chamber. The meniscus of the mercury should be marked. Another 0.1 ml. of water is added to the cup, and allowed to run in and mark the next mercury level. Continue in the same manner till mark 0.4 ml. is reached.

FIG. 4.—Preparation of reaction chamber capillary for calibration by weighing mercury.

Another method of marking is as follows. Before fusing the glass parts of the reaction chamber, part is weighed on an analytical balance and filled with mercury till the beginning of the uniform part of the capillary as shown in Fig. 4, then weighed again and marked. The weight of mercury divided by the specific gravity of mercury at a given temperature (e.g., 13.5388 at 23° C.) gives the volume of this space. The amount of mercury is adjusted so as to obtain a whole number and marked. Now the additional volume of mercury is added, representing 400 cubic millimetres and marked. The distance between the two marks is subdivided in 0.01 ml.

Reagents

Distilled Water.—To 100 ml. of distilled water 1 drop of caprylic alcohol is added and mixed well.

Lactic Acid Solution.—Concentrated lactic acid, 5 ml., and 10 g. of urea are placed in a 100-ml. measuring flask and a few drops of water saturated with phenol red added. The volume is made up to the mark with distilled water and 1 drop of caprylic alcohol is added. The solution is shaken well.

CO₂ Standard Solution.—Anhydrous sodium carbonate, 1.191 g., is dissolved in distilled water, stained red by adding phenol red, then made up to 500 ml. in a volumetric flask and stored under mineral oil. This solution corresponds to 50% CO₂.

Principle of the Method of Determining CO₂

Carbon dioxide is liberated from plasma or serum or standard sodium carbonate solution by adding lactic acid. The gas is liberated by shaking the mixture under reduced pressure and then its pressure (p_1) is measured at a constant volume. After expelling the gas the pressure (p_2) of the water vapour of the fluid is measured at the same volume. The value, $p_1 - p_2$, at the temperature, t , is then transferred to Van Slyke's nomogram and the percentage of CO₂ is read off.

Operating the Apparatus

Blank Determination.—About 0.4 ml. of water is introduced into the upper cup. The levelling bulb is lowered to position 2 so that the level of mercury in the levelling bulb is at the height of mercury at the 0.4 ml. mark in the extraction chamber and water allowed to run down to the 0.3 ml. mark through the stopcock (A). The remaining water is drawn off, and about 0.2 ml. lactic acid added and allowed to run into the chamber down to the 0.4 ml. mark. The cup is sealed with a drop of mercury which is dropped just below bulb E by lowering the levelling bulb, closing stopcock B, and replacing the levelling bulb in position 2. The apparatus is shaken for 30 seconds and the gas (water vapour) set free. The apparatus is shaken by holding the burette holder rod with one hand near its upper end, when it is tilted on the right back corner of the base, the other hand placed under the left front corner and the apparatus shaken using the rear corner as a pivot. The stopcock is opened till the mercury rises, then closed again. The levelling bulb is raised to position 1 (above stopcock A), stopcock B opened, then slowly stopcock A is opened and air expelled and some of the fluid till the mercury reaches mark 0.3 ml. The meniscus of the fluid is lowered to mark 0.3 ml. and the pressure, e.g., 105 mm., read on the manometer; this corresponds to the pressure of the saturated water vapour of the fluid. The manometer readings are repeated with the fluid meniscus at the 0.2 and 0.1 ml. marks, e.g., 112 and 118 mm. respectively. These readings give the p_2 values. The procedure is repeated several times till constant readings are obtained.

Determination of Standard.—Into the cup 0.1 ml. of water is introduced and under it 0.1 ml. of sodium carbonate standard with a "two-mark" 0.1 ml. pipette. The tip of the pipette should be held in the water. This method of introducing fluid to be tested prevents contact with air.

Stopcock A is opened and the liquid allowed slowly in, leaving always some fluid in the cup to avoid air entering, add distilled water and let it in until the fluid reaches mark 0.3 ml. About 0.2 ml. of lactic acid solution is added to the cup and allowed to run into the chamber to the 0.4 ml. mark and sealed with a drop of mercury. The mercury is lowered to the lower part of bulb E, stopcock B closed and the levelling bulb placed in position. The apparatus is shaken for 30 seconds to set free the gas. Stopcock B is opened and fluid raised to the 0.2 ml. mark. The pressure on the manometer is read, e.g., 312 mm., $p_1 - p_2 = 312 - 112 = 200$. Using Van Slyke's nomogram, 50% CO₂ at 22° C. corresponds to $p_1 - p_2 = 222$ mm. mercury. In order to adjust the manometric reading of the micro-apparatus to the above nomogram, the mercury in the manometer is raised to the 334 mm. mark; at this position $p_1 - p_2 = 334 - 112 = 222$ mm. The fluid meniscus in the gas extraction chamber arm is marked and this would correspond to 50% CO₂.

Air is expelled, as described under "blank determination," and p_2 read at the new volume mark. The determination of CO₂ is repeated as described above and

the mark corrected on the chamber capillary according to p_2 . This procedure is repeated three times. At the end of each determination gas is removed and the blank volume measured again. When the above procedure was employed it was found that the final mark for reading pressure was at the 0.175–0.180 ml. mark.

Another method for introducing fluid into the reaction chamber is as follows: The tip of a 0.1 ml. Ostwald pipette with a micro-stopcock is furnished with an extended polyethylene tube (Rappaport and Eichhorn, 1955) so that the capillary tip will not include air bubbles. The plasma or standard solution is introduced into the extraction chamber exactly as in the macro-apparatus using a 3:1 mixture of solutions a and b. The levelling bulb is placed in position 2; at this position the fluid can enter the reaction chamber capillary only to the 0.4 ml. mark. After the sample and lactic acid solution are introduced up to mark 0.4 ml. the procedure is continued as described above.

When no analytical balance or pure reagents are available for the preparation of the standard solution of sodium carbonate, the calibration may be performed by means of a plasma sample, the CO_2 content of which had been previously determined in a macro-Van Slyke apparatus.

The Test

Drawing of Blood.—Blood is obtained from the finger tip with the following precautions.

A capillary pipette of about 1.6 mm. diameter is filled with a mixture of NaF and potassium oxalate powder and the powder shaken out. A drop of paraffin oil is introduced into one end. The tip of the finger is punctured and coated with paraffin oil. The pipette with the paraffin plug is held under the finger as shown in Fig. 5 and the blood allowed to flow in, thus moving

FIG. 5.—Collection of blood from finger tip under paraffin oil.

the paraffin ahead of it. When the pipette is almost full the blood is expelled under paraffin oil into a centrifuge tube containing some NaF and potassium oxalate powder. The blood is mixed to prevent clotting

and centrifuged. The plasma is used for the test in the same way as a sodium carbonate standard. Readings on the manometer are transferred to nomograms and the percentage volume of CO_2 directly read off. The same pipette as for standardization should be used, otherwise discrepancies in the results might occur.

High CO_2 Content.—When the CO_2 content of plasma exceeds 80%, the manometric scale of the micro-apparatus does not suffice. In such cases the sample is examined again with a smaller volume of test-sample and the results multiplied by a proper factor. In order to avoid repetition of the whole test, the reading of p_1 and p_2 may be done at a predetermined lower pressure, e.g., one-third less than in the normal procedure; the mercury in the manometer would then be still within the scale. The reduced pressure will correspond to an increased volume of gas in the reaction chamber capillary. Therefore the position of a new mark for the increased volume must be found by taking a plasma sample of a known CO_2 content of about 60% corresponding according to Van Slyke's nomogram to a $p_1 - p_2$ of 269 mm. If one-third of this value is deducted, the resulting 40% CO_2 content corresponds to $p_1 - p_2$ of 188 mm. Therefore, after extraction of air the mercury level in the manometer is brought to 188 p_2 and the gas volume in the extraction chamber marked. It will be found to be about 0.25 to 0.26 c.mm. Air is expelled and p_2 read on the same mark. The examination is repeated and corrected according to p_2 . For calculating the results, as obtained at this new position, e.g., 0.25 c.mm., half of the result read on the nomogram is added; e.g., if the volume found on the nomogram equals 60% CO_2 content, then the actual CO_2 content is $60 + \frac{60}{2} = 90\%$. The same principle is to be applied

to examinations of 0.05 ml. quantities of plasma with a very low CO_2 content. Here, however, the pressure has to be increased by taking a smaller volume of gas, so that the resulting reading may be applied to Van Slyke's nomogram. This smaller volume is approximately 0.09 c.cm. for a 0.05 ml. sample and the exact determination of the position of the mark is performed as above.

When pressure is read at a gas volume of 0.175 to 0.180 c.mm. and the test sample is only 0.05 ml., the result read off the nomogram, not the pressure readings, has to be multiplied by a factor of 2.

The three marks 0.09 ml. (small volume), 0.175 ml. (medium volume), and 0.25 ml. (large volume) have to be used according to requirements. Normally 0.1 ml. plasma is used for examination and reading is done on the medium mark 0.175 ml. A very low alkali reserve (20% and less) with only 0.05 ml. plasma available should be read on the first mark 0.09 ml. (decreased volume), the result being read off directly on the nomogram. In the case of a very high alkali reserve (above 80%) the third mark 0.25 ml. (increased volume) should be used; to the value found in the nomogram half of its volume has to be added. These three marks are slightly different on each apparatus, but once determined their value is constant.

Cleaning the Apparatus

After each series of examinations the reaction chamber is cleaned with a 1% solution of triethanolamine stained with phenol red; this is followed by lactic acid water.

When the mercury becomes dirty and begins to form little balls in the reaction chamber 1 to 2 ml. of 20% ferricyanide solution is introduced into the reaction chamber and shaken well. Lactic acid and water follow as above.

Improvements

In a later model of the apparatus we introduced a magnetic stirrer,* which permitted vigorous mixing of the contents of the reaction chamber without the need of manual shaking. A twisted piece of stainless steel wire was enclosed in the reaction chamber and set into violent motion by means of a vibrator.

Summary

The apparatus described permits accurate determination of the CO₂ content of 0.05 ml. and 0.1 ml. samples of plasma. The calibration of the apparatus is so as to permit direct reading of the results from Van Slyke's nomogram.

REFERENCES

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Sixth International Blood Transfusion Congress and Sixth International Hematology Congress

This meeting will be held from Monday, September 3, to Wednesday, September 5, 1956, in Boston, Mass. The Sixth International Hematology Congress will meet from Monday, August 27, until Saturday, September 1, 1956. Joint programmes are planned for September 1 and 3. All meetings will be held at the Hotel Somerset, Boston, Mass.

International European Congress on Clinical Chemistry

The Second International European Congress on Clinical Chemistry will take place in Stockholm, Sweden, from August 19 to 23, 1957. Free papers will be read, but some topics will be reviewed by invited specialists. The Congress will be combined with an international exhibition of laboratory instruments.

Organizing committee: Bertil Josephson, chairman, St. Erik's Hospital, Stockholm; Kjell Agner, general Secretary, Serafimer Hospital, Stockholm; Bo Norberg, treasurer, Sabbatsberg's Hospital, Stockholm; Ingemar Jungner, exhibition manager, Epidemiologic Hospital, Stockholm. The address of the Congress is Box 12024, Stockholm 12.

European Society of Haematology

The sixth congress will be held in Copenhagen from Sunday, August 26, to Friday, August 31, 1957. Enquiries should be sent to Dr. A. Videbaek, Blegdamsvej 11, Copenhagen Ø, Denmark.

* Messrs. Thomas and Co. are now marketing the normal Van Slyke apparatus with a magnetic vibrator.

The Improved Determination of Iron in Serum

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Three main difficulties are encountered in the colorimetric determination of iron in serum as follows.

(1) Owing to the minute amount of iron present, a large sample of serum must be used and, even so, the sensitivity of most of the available methods is low. Using 22' dipyriddy, 1 : 10 phenanthroline, thiocyanate, or thioglycolic acid to develop the colour, it is usual to obtain an optical density of 0.03 to 0.04 on analysing a sample containing 100 µg. of iron/100 ml. using a final dilution of 1 in 4 and employing 10 mm. cells and the most favourable wavelength or filter. The range of optical densities between which the relative error is least is 0.2 to 0.7 (Archibald, 1950), and below an optical density of 0.1 the relative error increases rapidly. Recently 4 : 7 diphenyl 1 : 10 phenanthroline has been used for the determination of iron in serum (Peterson, 1953). This reagent gives a red colour with ferrous iron, having an optical density two to three times as great as that obtained using conventional reagents, and the iron complex is insoluble in water and can be extracted and concentrated from an aqueous medium by means of amyl alcohol or hexanol. Some of the reagents used in the determination can be rendered iron-free by preliminary extraction with this colour reagent. These advantages are offset by the tedium of extraction, which introduces an extra stage in an analysis which is already sufficiently complicated.

(2) It is difficult to determine whether all the iron has been extracted from the serum by the method of protein precipitation used. Even if all the iron added to serum is recovered, this does not prove that the iron originally present is being extracted completely.

(3) Iron present as haemoglobin may be released during the analysis and give false high values, particularly in methods which employ wet digestion or ashing to remove protein.

The object of the present investigation was to devise a reagent which would be as sensitive as 4 : 7 diphenyl 1 : 10 phenanthroline, but which would give a water-soluble colour with iron.

Experimental

The substance 4 : 7 diphenyl 1 : 10 phenanthroline is a very stable solid, insoluble in water, and should be capable of sulphonation without destroying its structure. Attempts at sulphonation were made, first with sulphuric acid and then with fuming sulphuric acid. Both attempts were partially successful, but the resulting derivatives, though giving an intense red with ferrous iron, were