The Astrup method of assessment of changes in the acid-base state of patients is a quick, reliable procedure, giving positive information of an unequivocal character. It involves obtaining the following values:

1. Actual pH of whole blood, anaerobically collected.
2. Whole blood pH, determined after equilibrating the sample with a carbon dioxide-oxygen mixture of known CO2 tension.

From the latter value (equilibrated pH) plus the haemoglobin value, by using a nomogram, may be ascertained the following:

- Base Excess or Deficit, Standard Bicarbonate and Buffer Base.
- If in addition, the former value (Actual pH) is obtained, then the following may also be ascertained:
  - pCO2, Total CO2, Actual Bicarbonate, and CO2 combining power.

Of these, the important values are:

1. Base Excess or Deficit - reflecting metabolic changes.
2. pCO2 - reflecting respiratory changes.

Since metabolic changes in the acid-base state are by far the commonest in general hospital work, it is found that most requirements are met by simply measuring the pH of the equilibrated sample of blood, estimating its haemoglobin level and referring to a nomogram.

Definitions as given by Astrup et al.

1. **Standard Bicarbonate** (6,7)
   This is the bicarbonate content of the plasma part of whole blood measured at 38°C, at a pCO2 of 40mm. Of Hg, with the haemoglobin fully oxygenated.
   Under these standardised conditions, the pH of the sample of blood is a direct indication of its bicarbonate content. The conversion of pH to standard bicarbonate is obtained either from the calibrated scale of the pH meter, the Henderson-Hasselbach equation, or from the nomogram of Anderson & Engel (1). Expressed in m.Eq/litre.

2. **Buffer Base** (1,8) (B.B.)
   The sum of the buffer anions, mainly bicarbonate and proteinate ions in m.Eq/litre.

3. **Base Excess** (B.E.)
   The difference between the Buffer Base found and the normal buffer base. Expressed as plus or minus (the latter indicating a deficit) in m.Eq/litre.

4. **pCO2**
   This is the partial pressure of CO2 in the blood at the moment of sampling. This is the measure of the respiratory component of the acid-base state, expressed in m.m. of Hg.

5. **Normal Buffer Base** (1) (NBB).
   The Buffer Base of blood with a pH of 7.38 at a pCO2 of 40 m.m. Hg.
   NBB=40.8 + 0.36x haemoglobin conc. (in g.per 100 ml.).
   As the standard bicarbonate does not give directly the amount in m.Eq/litre of fixed acid or base causing a change in the base content of the blood, the value of Base Excess is employed instead.

**PRINCIPLE OF THE ASTRUP METHOD**

If a graph is prepared on semi-log paper (logarithmic, 1 cycle x natural), showing the relations between pH and log pCO2 of a blood sample, the result is a straight line. The slope of the line is a function of the buffer capacity of the blood, and the position of the line is a function of the base content of the blood.

Once the line is obtained in any case, if the actual whole-blood pH is found, then the actual pCO2 can also be found. The nomogram of Anderson and Engel is such a graph (1).

The original Astrup apparatus employs equilibration with one gas mixture and requires a knowledge of the haemoglobin
level, while the new Ultra Micro Astrup Method employs equilibration with two samples and two different gas mixtures, and the haemoglobin value is not required.

I propose to outline the actual method of using the original Astrup apparatus, as employed in this laboratory.

APPARATUS

Astrup Macro Apparatus Type E50101 - made by Messrs Radiometer, Copenhagen, kept permanently warm via the water jacket at 38°C.

Water Bath - a plastic tank is suitable and cheap.

Thermocell - a reading accuracy of 0.005 pH unit is desirable. The combined electrode of the Radiometer Model 22 pH meter is designed to fit the Astrup apparatus.

Thermostatted circulating pump - The Techno Tempunit is satisfactory.

Ph Meter - a reading accuracy of 0.005 pH unit is desirable. The combined electrode of the Radiometer Model 22 pH meter is designed to fit the Astrup apparatus.

The Radiometer extension meter gives the required reading accuracy.

CO2/O2 Cylinders - ordered from N.Z. Industrial Gases Ltd., to contain 5.6% CO2 and 94.4% oxygen. The CO2 content must be known to within 0.05%, i.e., to two significant figures, and should be within the range of 5.4% to 5.8% CO2.

These mixtures are analysed by the Dominion Laboratory before being forwarded.

Reducing Valve - British Oxygen Co’s gas regulator valve is suitable.

Stoppered Tubes - for distilled water and standard buffer, filled and placed in rack in water bath at 38°C., five minutes before use.

Syringes - 10 ml., oiled and dry-heat sterilized in some sort of container to maintain sterility.

REAGENTS

1. Heparin Solution - 5000 units/ml.
   For adding to syringe
   Dissolve 200 mgms (== 10,000 units) of calcium heparin in 2 mls. Sterile distilled water, in sterile dropping bottle.
   Store in refrigerator.
   Use 1 drop per syringe.

2. Heparin - Fluoride Solution
   For Base Excess Bottle:
   Sodium heparin 10,000 units or 200 mgms. Powder
   Sodium Fluoride A.R. 2.5 grams
   Distilled water to 100 ml
   Place 0.5 mls. Of this solution (1 mgms. Or 50 units of heparin and 12.5 mgms. NaF) in bottle and dry in oven.
   For 5 mls. Blood.

4. Silicone Defoamer as purchased from Messrs Radiometer.

5. Potassium Chloride A.R. as fine crystals and as saturated solution.

6. Standard Buffer Solutions....
   A. M/15 primary potassium phosphate
      2.2695 gms of dry KH2PO4 (Sorenson salt, Merck) are dissolved in 250 mls. of deionized water.
      Refrigerate.
   B. M/15 Secondary Sodium Phosphate-
      11.8667 grams of Na2HPO4. 2H2O (Sorenson in 1000 mls. Of deionized distilled water salt, Merck) are dissolved.
      Refrigerate.
      Working Solution M/15 pH 7.380 ± 0.005 @ 38°C.
      Warm stock solutions to room temperature and mix 20 mls. of A with 80 mls. of B.

7. Paraffin Oil/Ether - equal parts neutralized paraffin oil and anaesthetic ether.
   The oil may be neutralized as follows:-
   In a 500 ml. separatory funnel place about 200 ml. of oil, an equal volume of distilled water, and a few drops of 0.1% phenol red solution.
   Add 0.02 N NaOH a drop at a time with vigorous shaking until the water solution of indicator becomes permanently pink.
   Centrifuge the oil to remove suspended water droplets.
   Decant the clear oil with care, and store in a stoppered bottle.

8. Deionised Water
   Distilled water is passed through a column of ion exchange resin. The resin employed is Biodeminrolit supplied by J.J. Niven & Co. Ltd.
   This process is quick, and removes CO2 from the water without the need to boil it.
   This water is hereinafter referred to as Water.
PROCEDURE
Immediately prior to performing an estimation, the pH meter is switched on to warm up, and adjusted to read the correct pH when the chamber of the Astrup apparatus is filled with standard buffer solution at 38°C.
After rinsing the chamber three times with water at 38°C, the instrument is left with the chamber filled with water, and is ready for use.

COLLECTION OF BLOOD
(1) When Base Excess or Standard Bicarbonate only are required:-
Venous blood is collected with any dry syringe without any particular precautions, except avoiding venous stasis as far as possible, and 4-5 mls. are placed in a specimen bottle containing dried heparin/sodium fluoride mixture, and shaken briefly. This specimen is suitable for analysis for an hour or two.

(2) When actual pH, in addition to Base Excess and Standard Bicarbonate is required:-
Venous blood from a preferably warmed arm is collected with preferably no stasis, in a well-fitting, oiled 10 ml. syringe to which has been added just before using one drop of a calcium heparin solution (5,000 I.U. per ml.).
This drop, plus the oil, leaves almost no air space in the syringe, thus assisting the anaerobic collection of the sample. Immediately on removing the needle from the vein, the syringe is pointed upwards, and any tiny air bubble is ejected into a cotton wool swab held over the needle point. A large drop of mercury is then sucked into the syringe from a small bottle containing 1-2 mls. The needle point is then pushed into an ordinary clean cork and the blood mixed thoroughly.
The actual pH should be determined as soon as possible.
Some 12-13 mls. of blood can be collected in a good 10 ml. syringe, and this provides sufficient specimen for actual pH, Base Excess or Standard HCO₃, Hb and P.C.V. and Na, K, Cl and N.P.N. estimations. (For the N.P.N. a small correction must be made, allowing for the nitrogen in the heparin. This may readily be found by experiment).
Just before the pH determination, the syringe is briefly warmed in hot water.

DETERMINATION OF ACTUAL BLOOD pH
The water is ejected from the chamber of the apparatus and mercury raised up to fill the bottom of the cup, which is then dried with a piece of folded filter paper. The specimen is thoroughly mixed in the syringe, the needle removed and replaced with a 1 inch piece of sphygmomanometer tubing (narrow bore and thick wall) which is immediately filled with blood.
As rapidly as possible, the syringe is inverted and the end of the tubing pressed firmly against the bottom of the cup, under the surface of the mercury.
By manipulating the taps, the mercury in the chamber is run down, drawing blood after it, until the chamber contains 2-3 mls. of blood-enough to cover the electrode. Both top and bottom taps are closed, the syringe removed and the needle and cork replaced on it. The pH meter is switched to the reading position and a reading made after about 1 minute.
The blood may then be raised into the cup and pipetted of for other tests, e.g., Hb and P.C.V., and N.P.N.
After rinsing the chamber 3-4 times with warm water, the pH meter setting is checked again with warm standard buffer. If necessary, the process must be repeated with more blood from the syringe, which may be kept in the water bath at 38°C, in the meantime. The chamber is finally left filled with water.
The remaining blood in the syringe is now distributed into specimen bottles as required, e.g., 3 mls. in Base Excess bottle, and 6-7 mls. in a centrifuge tube for plasma Na, K and Cl. estimations, if these are requested.

DETERMINATION OF EQUILIBRATED pH
The pH meter and apparatus are prepared as described above to the point where the mercury fills the lower part of the cup and the latter is dried.
About 2.5 mls. of blood from the ‘Base Excess’ bottle is poured into the cup, and a small portion of antifoam emulsion is smeared around the stem of the cup with a piece of glass rod.
Mercury and blood are then run slowly into the chamber, at the same time using the glass rod to help some of the antifoam emulsion to pass into the chamber also. This emulsion is like vaseline in consistency and very little is actually needed to prevent foaming and consequent haemolysis.
When the top of the mercury column is level with the lower edge of the side tube of the chamber, the lower tap is closed.
With the tap of the side arm closed, and the flow indicator tap turned to air, the gas cylinder tap is turned on, and by adjusting the reducing valve control, 3-4 bubbles per second are allowed to flow through the indicator. By turning the above-mentioned taps, the gas is caused to flow through the blood sample, and any necessary adjustment is made to the flow rate. If flow is to fast, blood is splashed about vigorously and haemolysis may occur, while if the rate is too slow, equilibration will not be complete in the desired time.
After about 2 minutes, the main cylinder valve may be closed, the reducing valve containing sufficient gas to last the
remaining three minutes. The life of the cylinder may thus be almost doubled (if the cylinder is nearly empty, the reducing valve may hold perhaps only 1 minute’s supply of gas, and the final portion is sprayed all over the room!)

After a total of 5 minutes’ bubbling, proceed as follows:-

Turn gas supply to flow to air from flow indicator, raise the blood to cover the electrode, close top and bottom chamber taps, switch pH meter to reading position, turn of gas at cylinder reducing valve, and read the pH after about half a minute.

The extension pH meter made by Radiometer is calibrated also in m. Mols. per litre of standard bicarbonate, and this may be read of directly, provided the pCO₂ of the cylinder gas is 40 mm. Hg. or close to it.

The blood is then pipetted off and the pH meter buffer again checked. If necessary, the equilibrated pH may be determined again on the same blood sample. The chamber is left filled with water and the pH meter switched off. As the whole apparatus is kept constantly at 38ºC. it is always ready for use.

CORRECTIONS
1. The pCO₂ of the cylinder gas is found according to the following (3):-

   \[ \text{pCO}_2 = \frac{(B-W) \times \text{per cent CO}_2}{100} \]

   where B == barometric pressure in m.m. Hg., and W is the vapour pressure of water at 38ºC. (==50 m.m. Hg.).

2. If the pCO₂ thus found deviates from 40 m.m. Hg., and is between 36 and 44 mm. Hg., it may be convenient, in using the nomogram, to correct the equilibrated pH as follows (6):-

   \[ \text{PH}_{40} = \text{pH measured} + 0.006 \times (\text{pCO}_2 \text{ used} - 40) \]

   It is helpful to prepare tables in each case for quick reference. A table should also be prepared relating Normal Buffer Base and haemoglobin concentrations at 0.5 grams per cent intervals.

USING THE NOMOGRAM OF ANDERSON AND ENGEL
In the procedure described, when equilibration is carried out with a single CO₂/O₂ mixture, the Haemoglobin value is required to obtain the Normal Buffer Base of the sample. We thus have the following items for use with the nomogram:-

- Normal Buffer Base, Actual pH, and Equilibrated pH.

The Normal Buffer Base gives the slope of the line to be drawn, the equilibrated pH gives the position of the line, whence the Base Excess may be directly obtained, and the point on the line provided by the Actual pH gives the pCO₂ of the sample.

When the Ultra Micro Astrup method (2) of analysis is performed, equilibration is carried out at two different CO₂ tensions and the position and slope of the line obtained, without the need for knowledge of the haemoglobin value. This method employs capillary blood, is quicker to perform than the original macro method described here and is thus eminently suitable for serial determinations, especially in following the respiratory component of the acid base metabolism.

In practice, using the macro method the whole procedure of obtaining actual pH and Standard Bicarbonate and Base Excess figures takes 12-13 minutes total elapsed time. If Standard Bicarbonate or Base Excess only are required, as is usually the case here, only about 5 minutes’ work is involved. During equilibration other work may be proceeded with.

The appended references cover the work which has been done in the course of the development of this new method of investigation of acid-base state up to the present time.

REFERENCES