Mathematics and Physics for Anaesthetists at the Institute of Basic Medical Sciences at the Royal College of Surgeons of England: Programme 6: Blood – Gas Analysis

Presented by DW Hill, Reader in Medical Physics.

University of London Audio-Visual Centre, 1977.

Film extract by courtesy of Dr JT Wright, Bio-engineering and Medical Physics Unit, University of Liverpool.

Produced by David R Clark; Michael Tomlinson.

Made for British Postgraduate Medical Federation.

Black-and-white
Duration: 00:24:37:06

00:00:00:00

<**Opening titles**>

<**DW Hill over film of surgery in an operating theatre** >

The measurement of blood gasses and the pH of a patient's blood is often performed during anaesthesia, intensive care and in respiratory function studies. Blood gas measurements usually consist of the determination of the partial pressures, that is to say, tensions of oxygen and carbon dioxide in a sample of arterial blood. Since these measurements may be required in a hurry at any hour of the day or night, it is important that the apparatus should be simple to use and able to be calibrated by junior staff at short notice.

<**Hill to camera**>

The past decade has seen a steady increase in the ease of use and reliability of electrode systems designed for the measurement of blood carbon dioxide and oxygen tensions and blood pH. As anaesthetists you need to be familiar with the operating principles of the electrodes, how to take and store blood samples for them and finally how to calibrate and actually use equipment.

<**Hill over tables showing various pH levels**>

The concept of pH has been discussed in another videotape in this series, Logarithms and Exponentials for Anaesthetists. In simple terms, the pH value of a particular solution is a measure of its acidity or alkalinity. The pH is defined as the negative logarithm of the hydrogen ion concentration of the solution. That is, $pH= -\log_{10}[H^+]$. Thus, a range of hydrogen ion concentrations from 1 to 10^{-14}g per litre could be encompassed within a pH range of 0 to14.

Normal values of blood pH are 7.35 to 7.45 for arterial blood and 7.32 to 7.42 for venous blood, both at 37˚C. The concentration of hydrogen ions in blood is often expressed in terms of nM per litre so that a typical arterial pH is equivalent to a hydrogen ion concentration of Antilog -7.4, that is $3.98x10^{-8}$ M per litre, which is the same as 39.8 nM per litre.

An electrode potential arises at the interface between two material phases, it develops because the rate of passage of ions across the interface is different for every ion, and once across, the ions may combine with electrons to form atoms. The most common case is the exchange of one kind of metal ion in solution for a second, initially present as atoms in a rod. If the ions enter the metal faster than the metal dissolves, an excess of negatively charged ions develops in the solution and the metal becomes positively charged by the loss of electrons that neutralised the incoming ions.

<**Hill over series of animated models referring to electrode potentials**>

An electrode potential is also developed when an interface is produced by introducing a semi-permeable membrane between two liquid phases. If the membrane is such that ions pass more freely in one direction than the other, then an equilibrium will be reached that has separated positive and negative charges. When this equilibrium is attained across the membrane, the potential developed across it is proportional to the logarithm of the ratio of the concentration of the ions on either side to which the membrane is selectively permeable. The development of glass membranes which are selectively permeable to hydrogen ions has led to the widespread use of glass electrodes for the measurement of the pH of body fluids and also of blood carbon dioxide tensions.

In order to be able to compare the electrode potentials developed at various interfaces, a reference value is needed. And since these electrode potentials depend on the logarithm of the concentrations, the relative potentials are nearly pH values. A pH meter consists of one of these stable reference electrodes plus an indicator electrode placed in the solution under examination, and a means of measuring accurately the potential difference developed between the two electrodes. At its simplest, the electron system behaves like a battery whose open circuit voltage is pH dependent. The so-called battery has a very high value of internal resistance so that in order to be able to measure this true electromotive force, the meter must draw a negligible current from the electrodes.

Here you see a bulb-shaped, glass pH electrode forming part of a pH meter. The thin-balled bulb of special pH sensitive glass contains a buffer solution, usually of pH1 which constitutes a known stable value of pH. A chlorided silver wire is immersed in this buffer and is connected by a well-insulated screened cable to the input of a very high resistance mV meter. The circuit is completed back to the solution by means of a calomel electrode and a salt bridge. These are interfaces that have potential differences of their own, but which are stable, and do not vary with pH. This is also true for the chlorided silver wire which is really a silver/silver chloride electrode.

00:06:18:20

When the bulb or the glass electrode is immersed in the sample solution, a DC potential difference develops between the bulb's inside and outside surfaces. Hydrogen ions diffuse out of the bulb faster than they enter. After a short while the net negative charge left behind in the bulb holds back ions and an equilibrium is obtained when the rate of entry is the same as the rate of exit. The potential difference then reflects the hydrogen ion concentration outside the bulb.

A calomel electrode is a solid liquid interface. The bottom end consists of either an asbestos fibre or a plug of ceramic. It forms a liquid junction between the saturated KCL solution inside the electrode and the sample outside. The body of the electrode acts as a reservoir for saturated KCL solution and into this is immersed a cylinder of calomel mercurous chloride. A blob of mercury and a platinum wire enable contact to be made with the calomel, the wire being connected to the pH meter's input. The calomel electrode must also be thermostated. The ceramic plug allows current to pass but stops contamination or dilution of the KCL by the sample.

<**Hill over film showing practical version of pH electrode pair**>

This is a practical version of a pH electrode pair. On the left of the picture is the pH electrode, the blood passes through the centre of it as we shall see in a moment. On the right is its companion reference electrode. These electrodes, together with two more to measure blood PCO_2 and PO_2 are mounted in a thermostated bath to the right of this commercial fully automatic blood gas and blood pH analyser. This machine is designed to require only small amounts of blood and so the blood pathways are miniaturised.

The pH glass electrode fits into its socket, forming a continuous tube for the blood to pass through, and you can see the miniature pathway underneath the electrode and the reference electrode is immersed in saturated KCL solution and is now connected

to the blood sample via this salt bridge – you can see it running across horizontally there to the blood sample.

The output voltage from a glass electrode calomel reference electrode combination is normally zero when the sample is neutral, that is, has a pH value of approximately 7. For acid solutions the output goes positive and for alkaline solutions it goes negative. The internal resistance of a glass pH electrode is about 100 to 1000 megohms so that the input resistance of the pH meter must be at least 100,000 megohms to avoid loading the electrode by more than 1%.

00:09:26:00

<**Hill over graphs and film demonstrating accurate measurements of blood gas using pH meter**>

A typical range for a blood pH meter is 6.000 to 8.00 pH units and the meter must be able to detect a change of 1000th, that is 0.001 pH units. To permit calibration, the pH meter is provided with balanced and sloped controls. The balance control simply adds a variable voltage in series with the pH electrode system and allows the meter's output to be adjusted to correspond with the pH of a known buffer solution used for calibration. The slope control is an amplifier gain control which adjusts the slope of the calibration line, that is, the millivolts output per pH unit. All pH measurements are made by comparing the unknown pH with standard buffer solutions which have accurately known pH values. Suitable buffers are the two phosphate buffers which have pH values of 6.840 and 7.384 at 37˚C respectively.

In order to bring the buffers to 37˚C, they are passed through metal heat-exchanger spiral coils immersed in the water bath. Once poured from their storage bottles buffer solutions should never be returned. After pouring, the top of the bottle should be dried immediately and the top replaced to prevent contamination. The meter is set to read a pH of 6.840 at 37˚C on the first buffer, by means of the balance control, and then the slope control is used to obtain a reading of 7.384 on the second buffer.

The glass electrode is carefully rinsed with distilled water between the buffers. In this fully automatic analyser, depressing the calibration buttons starts the calibration process for each buffer in turn.

<**Hill to camera then over demonstration of correct method of taking blood for pH testing**>

Arterial blood is to be preferred for acid base balance studies and is essential if the sample is to be used to check on the adequacy of oxygenation. For accurate blood pH and gas tension measurements, glass syringes, which have first been acid washed, thoroughly rinsed and then autoclaved, should be used. Disposable plastic syringes may be convenient for routine clinical measurements but may produce errors in oxygen tension if prolonged storage is involved.

Syringes of 2 or 5ml capacity are suitable and the interior should have been thoroughly wetted with heparin solution which should only remain in the dead space of the syringe in the needle. The blood sample should be carefully drawn under anaerobic conditions and the syringe quickly capped with a metal cap containing clean mercury. The filled syringe should be stored in ice water to reduce metabolic changes if the measurements cannot be made immediately. It is very easy to lose high oxygen tensions by metabolism and contact with the air.

<**Hill over table listing procedures for non-automatic blood gas analyser machines**>

After calibrating the pH meter, the cuvette should be flushed with air and the first portion of the blood sample introduced. When the meter reading has steadied, the second aliquot is introduced and the meter read. Finally, the third portion is introduced and the average of the second and third readings is taken. The cuvette is then carefully rinsed out and left in a clean state ready for the next sample.

<**Hill over film demonstrating automatic blood gas analyser machine**>

In this automatic analyser, pressing the tip button causes the sampling tip to protrude. In this case a venous blood sample is first well agitated and then offered up to the sampling tip. Now, pressing the sampling button causes the sample to be sucked into the analyser. The blood first passes between the PO_2 and PCO_2 electrodes and then through the pH electrode.

In this analyser there are two other electrodes, their tips form the opposite sides of a cuvette. This is the PCO_2 electrode. The CO_2 tension in the blood is closely related to the blood's pH value. Numerically the value of the carbon dioxide tension of whole blood is the same as that of the partial pressure of the gas mixture with which the blood is in equilibrium.

<**Hill over table listing blood gas comparisons**>

Consider a dry gas mixture at a barometric pressure of 750mm of mercury and containing 5% by volume of carbon dioxide. The partial pressure of CO_2 in the mixture is equal to 5% of 750, that is 37.5mm of mercury from Dalton's law of partial pressures. If this gas mixture is equilibrated at 37˚C with blood in a thermometer, the partial pressure of CO_2 in the gas is given by 5% of, now, 750 minus 47, that is 35.2mm of mercury since the partial pressure of water vapour at 37˚C = 47mm of mercury. Once equilibrium has been obtained, the tension of CO_2 in the blood also equals 35.2mm of mercury.

<**Hill over film demonstrating a PCO_2 electrode**>

Direct reading PCO_2 electrodes are now available. The PCO_2 electrode is a pH sensitive glass electrode, arranged to measure the pH of a thin film of sodium bicarbonate solution which is localised in front of the electrode by this fabric mesh. The solution is separated from the blood sample by a Teflon membrane which is permeable to molecules of carbon dioxide but not to ions which might alter the pH of the bicarbonate solution. The second O ring holds this membrane in position. When the electrode is in place, its tip just protrudes into the cuvette.

A combination pH glass electrode and reference electrode is in contact with the bicarbonate solution. Carbon dioxide diffuses across the membrane in either direction depending upon the difference in partial pressures which exist across the membrane. Hydration of CO_2 in the water of the bicarbonate solution produces carbonic acid and changes the solution's pH. The output from the pH electrode system is logarithmically related to the blood PCO_2. A tenfold increase in PCO_2 is nearly equivalent to a reduction of 1 pH unit. An anti-logarithmic amplifier is employed to provide a linear scale in terms of PCO_2.

A typical PCO_2 meter would have two ranges: 1 to 260 and 10 to 2600mm mercury with a claimed accuracy of plus or minus 1mm mercury plus 1%. And the reproducibility is claimed to be plus or minus 0.2% at a PCO_2 of 100mm of mercury.

00:17:34:00

<**Hill over film demonstrating PCO_2 glass electrode**>

The most accurate method for calibrating a PCO_2 electrode is to tonometer blood to known CO_2 tensions in a thin film type tonometer. A calibration gas is passed over a thin film of blood formed by centrifugal action in the intermittently rotating tonometer. This technique obviates any problems due to a difference between blood and gas calibrations – the so-called blood-gas difference. The electrode can also be calibrated with known gas mixtures which have been humidified and warmed to 37˚C before entering the electrode cuvette. The gas mixture which is normally used to simulate the PCO_2 of whole blood is 5% carbon dioxide, 12% oxygen and 83% nitrogen. The mixture used for adjusting the slope of the calibration line is 10% CO_2 and 90% nitrogen. The combined effects of temperature changes upon the sensitivity of the pH electrode and the PCO_2 of the blood sample produce a total temperature coefficient for the PCO_2 electrode sensitivity of about 8% per ˚C. Thus, the electrode should be thermostated at 37˚ plus or minus 0.1˚C.

The other side of the analyser's cuvette is formed by the oxygen electrode. The maintenance of an adequate oxygen tension in the patient's blood is vitally important and hence, there's been an urgent requirement for a reliable oxygen electrode which can be used regularly with whole blood. If blood at 37˚C is equilibrated with a mixture of 5% carbon dioxide, 12% oxygen and 83% nitrogen, the oxygen tension is 12% of 75 minus 47, that is 84.6mm of mercury when the barometric pressure is 750mm of mercury.

The glass electrode used for pH and PCO_2 measurements is essentially a voltage-generating device, whereas the polarographic oxygen electrode is a current-generating device.

<**Hill over diagram detailing function of polarographic oxygen electrode**>

It is provided with a constant polarising voltage of approximately 600 millivolts and produces an output current which is directly proportional to the oxygen tension diffusing to the active surface of the electrode. The potentiometer derives approximately 600 millivolts, that is 0.6 volts from the 1.3 volt mercury battery, and applies this across the polarographic cell which consists of a platinum cathode and a silver/silver chloride reference electrode. For a relatively large cathode, the current that could be recorded on a sensitive galvanometer. Each molecule of oxygen reaching the cathode reacts with 4 electrons and this electron flow is measured with the galvanometer. As a result of the cell operating, a constant zone of diffusion is established in front of the cathode. In the larger electrodes it was necessary to agitate the blood sample in order to prevent a local depletion of oxygen adjacent to the electrode. But with modern low-current electrodes this is no longer necessary.

<**Hill over film showing oxygen electrode**>

Protein deposits from whole blood on an oxygen electrode can cause it to be poisoned. This problem was overcome by Clarke in 1956 who placed both anode and cathode behind a single Teflon membrane which allowed oxygen to diffuse through but held back the blood. You can see the membrane held in place by the O

ring. The calibration of a PO_2 electrode is similar to that for a PCO_2 electrode, tonometered whole blood being the most accurate method. The same two mixtures can be used for a gas calibration as are used for the CO_2 electrode; that is, 10% carbon dioxide and 90% nitrogen; and 5% carbon dioxide, 12% oxygen and 93% nitrogen. The first mixture is employed for setting the zero of the meter and the second to set up the meter on a known tension. Modern high gain amplifiers have allowed the miniaturisation of all 3 electrodes, pH, PCO_2 and PO_2 so that they can be mounted inside a common cuvette and a blood sample of only 0.4ml is required to cover all 3 determinations.

A typical operating range for a PO_2 electrode is 0 to1600mm of mercury with a quoted accuracy of plus or minus 1mm of mercury plus 1%, and a 97% response time at 37˚C of 30 seconds.

00:22:37:00

<**Hill over film demonstrating automatic and manual blood gas analysers**>

In many hospitals blood gases are measured in the chemical pathology laboratory which must handle a large number of samples on a 24 hourly basis. This requirement has led equipment manufacturers to develop fully automatic analysers which have automatic calibration, flush and sample cycles. Such an analyser is the model 613 by Instrumentation Laboratory. It can handle 30 blood samples per hour and only needs 0.35ml per sample of blood. Automatic monitoring circuits indicate any failure of the PO_2 and PCO_2 electrode membranes. The 4-digit display is provided for pH, PO_2 and PCO_2, and the built-in calculator also gives the derived values for the actual bicarbonate, carbon dioxide content, base excess and standard bicarbonate and can print out all these values if required.

In Cipla manual analysers, it is important to flush out the blood pathways prior to taking in the next blood sample.

<**Hill to camera**>

The measurement of blood gas tensions and blood pH can be of vital importance in the management of a patient during anaesthesia or intensive care. Hence, it is essential that the blood sample should be correctly drawn and the apparatus should be well-maintained and calibrated. These are matters with which you should be familiar.

<**End credits**