Measurement of arterial and capillary blood oxygen tension

J. H. JOHNSTONE
From Queen Elizabeth Hospital, Birmingham

SYNOPSIS An oxygen electrode system, supplied as an attachment to the Radiometer Astrup micro equipment for blood pH determination (AME I), has been investigated. Determination of blood oxygen tension using this electrode system has been compared with tension measurements using an established Bishop type oxygen electrode and satisfactory agreement was found. The storage of blood for routine estimation of oxygen tension has been investigated. Capillary blood oxygen tension has been measured and compared with that of simultaneously taken arterial blood samples.

The first electrometric measurements of blood oxygen tension in biological fluids were made by Baumberger (1938) using the dropping mercury polarographic technique. Baumberger applied a potential to an electrolytic cell and showed that the resultant current was proportional to the amount of oxygen reduced at the cathode. As the negative potential of the cathode increased, the current increased, the current became limited by the rate of diffusion of oxygen to the cathode: a plateau was then reached, the height of which was proportional to the concentration of dissolved oxygen. There were practical difficulties in using this type of electrode; it became contaminated with protein and variations in the mercury drop size produced unreliable results.

A platinum cathode and an external reference anode of silver were first used in biological problems by Davis and Brenk (1942). The platinum cathode was mounted within a narrow glass tube which was applied to the biological solution. In an attempt to prevent the poisoning of the surface of the platinum the originators filled a small recess at the end of the electrode with saline and subsequently with agar. The principle of this type of solid electrode system was that if a solid cathode, such as platinum, was maintained at about -0.6 volts with respect to a non-polarizable anode, the current which flowed as a result of reduction of oxygen was proportional to the oxygen content of the solution.

In all these electrodes a major source of error was the contamination of the platinum electrode by chemical constituents present in the samples and a consequent reduction of the current generated by the electrode. Clark (1953) isolated the platinum electrode from the environment of the solution by placing it behind a cellophane membrane and this principle is used in all recent designs. However, as this type of membrane was permeable to water-soluble material, the problem of contamination was not completely solved.

In a further attempt to prevent poisoning of the platinum electrode, Clark (1956) proposed that the platinum electrode and the anode should be incorporated in a solid cell and that the exposed electrode end be isolated from the blood or fluid by a hydrophobic membrane. The use of a hydrophobic membrane solved the problem of poisoning of the platinum electrode. However, an inherent problem of a covered electrode system is the creation of an oxygen tension gradient within the liquid in contact with the membrane material.

In attempting to reduce the effect of the oxygen tension gradient many workers reduced the area of the platinum electrode. However, as the sensitivity of the electrode is proportional to the area of the platinum this had the effect of reducing the resultant current. Severinghaus and Bradley (1958) and Bishop (1960) introduced stirring of the blood sample by a rotating paddle and by magnetic stirring respectively, while Kreuzer, Watson, and Ball (1958) moved the electrode mechanically. The introduction of stirring devices created a marked increase in the size of the measuring chamber and necessitated much larger samples.

Another approach to the problem was to reduce the permeability of the membrane to oxygen and thus limit the relative importance of the oxygen gradient. An oxygen cell employing such a membrane would have a low current output for the same
external oxygen tension, so that a more sensitive recorder and amplifier would be required. The time required for sufficient oxygen to diffuse through and reach a steady maximum state would be prolonged. However, this would enable the measurement of oxygen tensions in unstirred samples (Polgar and Forster, 1960). This principle has been utilized in the unstirred electrode system manufactured by Radiometer, which is used in the present study.

The relationship between arterial and capillary blood levels of oxygen saturation has been investigated by Sigggaard Andersen, Jorgensen, and Naeraa (1962) and shown to be the same. However, the relationship of the arterial oxygen tension to that in capillary blood has not been reported.

This investigation tests the validity of the Radiometer unstirred oxygen electrode system, using an established electrode system for comparison, and the precautions necessary in the routine use of the oxygen electrode have been investigated. In addition, determinations on capillary blood have been compared with those on arterial blood collected at the same time.

METHODS

APPARATUS The oxygen electrode (Type E 5045) used was that supplied by Radiometer, consisting of a 20 μ diameter platinum cathode sealed into a glass cone and a silver ring anode. The electrodes are placed in an electrolyte solution behind a hydrophobic membrane which is mounted on the tip of the electrode and fastened by an o-ring (Fig. 1).

The electrolyte solution is a phosphate buffer to which some potassium chloride has been added to stabilize the potential of the anode.

The electrode is mounted within a Radiometer thermostated oxygen cell, D 615, as illustrated in Figure 2. The water inlet and outlet ports of the oxygen cell are connected to the thermostated water supply of the calomel electrode of the Radiometer micro pH equipment A.M.E.1.

The polarizing voltage of 700 mv. is supplied from the oxygen monitor (PHA 928) connected to the A.M.E.1 equipment and this monitor also converts the current produced into a voltage. This enables the expanded pH scale of the A.M.E.1 to be used for direct reading of oxygen tension. When this scale did not include oxygen calibration, for convenience the pH 7.0 point of the scale was always used for zero oxygen tension and the pH 8.0 point for 100 mm.Hg, although this could be varied according to the range required.

When the electrode was used for small amounts of blood, micro-inserts were fitted into the inlet Luer-lock fitment (I) and the outlet Luer-lock fitment (O) of the oxygen cell (Fig. 2).

The Radiometer micro pH suction equipment was modified for use with the micro inserts. A three-way plastic tap was attached to the inlet side of the waste blood trap, which allowed suction to be directed either to the micro pH electrode or to a suction line which was attached to the outlet micro insert (Fig. 2) of the oxygen cell. A glass T-piece was inserted in this suction line to facilitate the control of suction through the oxygen cell. A micro plastic capillary was attached to the inlet micro insert (I, Fig. 2). The controlled, anaerobic, transfer of blood from the capillary tubes in which it was taken to the oxygen cell was found to be more efficient if the plastic capillary was inserted into the blood capillary tube rather than inserting the blood capillary tube into the holder supplied by the manufacturer. All waste blood and calibration solutions were collected in the waste blood bottle of the A.M.E.1 apparatus.

MEMBRANE MATERIAL. Polypropylene or Viscose film was used in this study. The characteristics of these hydrophobic materials were such that the time required for 95% of the maximum response to a blood oxygen tension was 30 seconds at 38°C. The 95% maximum response time for water was 15 seconds.

Care was exercised with the polypropylene material because it was found to stretch easily. The Viscose membrane material was easier to handle.

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ELECTROLYTE SOLUTION The electrolyte solution used was that recommended by Radiometer and contained:—

5·1 g. Na₂HPO₄·2H₂O,
2·5 g. KH₂PO₄,
1·0 g. KCl,
and water to 100 ml.

PROCEDURE FOR USE OF THE OXYGEN ELECTRODE A strict daily procedure was adopted to ensure reproducible results.

A new piece of membrane was mounted on the electrode each day by the following procedure. (The membrane has a useful life of about 24 hours, then it deteriorates.) The o-ring was pushed onto a special mounting tool (Radiometer D 649) and held vertically in the left hand with the concave end uppermost. A 4 × 4 cm square of membrane material was placed centrally on top of the mounting tool and a drop of the electrolyte solution was placed directly above the concave area of the tool. The electrode, held vertically in the right hand, was then dipped into the drop of electrolyte solution and gently rotated to dislodge any air bubbles. The concave end of the tool was then pressed over the top of the electrode and the o-ring pushed into the recess in the electrode. The corners of the membrane were then pulled until the membrane fitted snugly without any creases over the tip of the electrode. Excess membrane was trimmed back to the o-ring.

When the Viscose film was used it was necessary to wait 15 to 20 minutes before a reading of oxygen tension could be obtained. The polypropylene film only required a period of five minutes.

CALIBRATION OF ELECTRODE The relationship of oxygen tension to the voltage reading on the scale was shown to be linear and therefore only two reference calibration points were required: a solution of zero oxygen tension and a solution of known oxygen tension.

The zero oxygen tension solution was made from 0-01M. sodium borate solution containing freshly added sodium sulphite (approximately 0·1 g./100 ml.).

The solution of known oxygen tension was water + KCl equilibrated with air at 38°C., the most convenient source being that in the water bath used for circulating the A.M.E.1 equipment.

The oxygen tension was calculated from

\[ PO_2 = (BP - V.P.) \times \frac{20·93}{100} \text{ mm.Hg} \]

Where B.P. is the barometric pressure in mm.Hg, V.P. is the vapour pressure (mm.Hg) of water at 38°C., and 20·93 is the volumes percentage of oxygen in atmospheric air. The water and air should be allowed to equilibrate for one hour after the water reaches 31°C. before a sample is used for calibration.

Calibration of the electrode in respect to zero oxygen tension and the other known oxygen tension was made as soon as the membrane became responsive. The calibration solution was withdrawn from the appropriate container by a glass syringe fitted with a length of narrow gauge plastic tubing. The plastic tubing was disconnected and any air bubbles were expelled from the syringe. The syringe was then attached to the lower Luer-lock fitting (Fig. 2), and the solution gently injected into the oxygen cell. A volume of fluid (0·5 ml.) corresponding to at least twice the total cell capacity was allowed to run to waste before a reading was made. During all measurements the syringe was left attached to the oxygen cell until maximum response was measured. Between calibration of the electrode with the zero oxygen tension solution and the air-equilibrated water, the oxygen cell was rinsed with 5 ml. of water from the thermostat bath of the A.M.E.1 to remove all traces of reducing agent.

Before the oxygen tension of an unknown sample was determined the calibration of the electrode was checked with a sample of air-equilibrated water. The cell was then rinsed with 5 ml. of 0·9% saline at 38°C. or water at 38°C. before the syringe containing the heparinized blood or unknown solution was attached to the lower Luer-lock fitting. The blood or fluid was injected into the oxygen cell with a force similar to that used for the injection of the calibration solution. At least one cell volume of blood or fluid (0·25 ml.) was allowed to flow to waste. The syringe was left in position until the maximum response was obtained and the oxygen tension read. The cell was then thoroughly rinsed with saline at 38°C. before a further sample of blood or fluid was introduced.

As a precaution against bacterial contamination, the oxygen cell and components were washed and stored in Hibitane when not in use.

PROCEDURE FOR MEASUREMENT OF CAPILLARY SAMPLES OF BLOOD The calibration of the oxygen electrode for use with capillary samples of blood was made with the micro inserts fitted in the inlet and outlet positions. Samples were sucked into the cell, and similar suction pressures were applied to the calibration solutions and the unknown samples.

The polythene capillary attached to the inlet insert (I, Fig. 2) was introduced into the body of the glass syringe containing the calibration solutions and sufficient fluid was drawn through the cell into the waste limb to ensure complete filling of the measuring chamber and the outlet insert, the side arm of the T-piece being used to control the suction. Time was allowed for maximum response before adjusting the calibration or before readings were made. Several cell volumes of either water or saline (at 38°C.) were sucked through to prevent contamination with the next sample.

Blood samples contained in heparinized capillary tubes (80 µl. capacity) were transferred to the electrode by inserting the tip of the polythene capillary into an end of the glass capillary and applying gentle suction. Because more than one capillary of blood had to be used the final 5 mm. of the blood column in the heparinized capillary was not transferred as a precaution against the introduction of an air column when the next capillary was attached to the polythene capillary.

The capacity of the measuring chamber, the inlet and outlet tubes was 0·25 ml. When adapted for use with micro blood samples the capacity of the polythene capillary, the inlet and outlet inserts, and the measuring chamber was 160 µl.

 Chlorhexidine 0·5%.
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COLLECTION OF BLOOD SPECIMENS  The glass syringes used for arterial blood samples contained a solution of heparin to inhibit clotting and to fill the dead space in the syringe.

Capillary samples were obtained by ear-lobe puncture. The ear lobe of the patient was treated with Trafuril1 to achieve maximum arterIALIZATION of the capillary circulation for five minutes before the puncture. The least possible pressure was applied to the ear to obtain the blood sample which flowed directly into the capillary tube. Any surface gathering of blood was removed and not allowed to flow into the capillary tube.

A minimum of two capillary tubes (2 × 80 μl) of blood was obtained from each patient. The ends of the tubes were immediately sealed with plasticine to prevent further contact with atmospheric oxygen. If the measurement of the blood oxygen tension could not be made immediately the capped syringe or the capillaries were stored in iced water.

COMPARISON OF RESULTS WITH THOSE OBTAINED USING THE BISHOP ELECTRODE  Blood samples throughout the range of oxygen tensions 15 mm. to 230 mm.Hg were obtained by arterial puncture or by artificial equilibration of blood samples with gas mixtures of known oxygen tension at 38°C. Each sample was analysed twice using both the Bishop (1960) electrode system, which had been calibrated against blood equilibrated with known gas mixtures, and the Radiometer oxygen electrode.

The relationship between values of the oxygen tensions for the two electrodes is shown in Figure 3. The regression coefficient was 1-0001 with the 95% confidence limits of 1-0188 and 0-9827.

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FIG. 3 The relationship of $P_{O_2}$ values of duplicate samples of arterial blood measured simultaneously on a Bishop type oxygen electrode and on the Radiometer oxygen electrode. The regression coefficient = 1-0001 with the 95% confidence limits of 1-0188 and 0-9827.

PRECISION OF THE METHOD  Three samples of blood at different levels of oxygenation were sampled anaerobically in glass syringes, capped, and immediately plunged into iced water. Each of the blood samples was rewarmed to 38°C and the blood was transferred anaerobically to capillary tubes, which were sealed to prevent contamination with atmospheric oxygen. The oxygen tension was measured on the samples of blood contained in capillary tubes by the method we adapted for small samples. The mean oxygen tension and the standard deviation for the three samples of blood are shown in Table I.

TABLE I

<table>
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<tr>
<th>Mean oxygen tension (mm.Hg)</th>
<th>No. of estimations</th>
<th>Range (mm.Hg)</th>
<th>S.D.</th>
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<td>31-1</td>
<td>109-1</td>
<td>137</td>
<td>0-2</td>
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<td>107-5 to 113-0</td>
<td>136-0 to 139-0</td>
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<tr>
<td>32-0</td>
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<td>1-2</td>
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STORAGE OF BLOOD SAMPLES  In the routine measurement of blood oxygen tension the method of storage of blood is of prime importance. A rapid fall in oxygen tension and saturation of blood has been shown by Severinghaus (1958), Nunn (1962), and Siggaard Andersen (1961). In the present investigation the effect on the oxygen tension of keeping blood at 22°C. (room temperature) and in iced water was studied in an attempt to find the most suitable conditions for the storage of blood while in transit from the patient to the electrode.

Blood which was artificially oxygenated by tonometry at a known oxygen tension at 38°C or obtained by arterial puncture was sampled in heparinized glass syringes and immediately capped. The initial measurement of the oxygen tension at 38°C was made at a known time interval, usually less than five minutes, after taking the sample. The samples were then maintained anaerobically at 22°C with occasional rotation. At selected times the oxygen tension was determined in duplicate at 38°C.

The alteration of blood oxygen tension with time at three different starting levels of oxygenation is shown in Figure 4.

The zero time levels were obtained by extrapolation to the ordinate. Each blood sample showed a rapid fall in the oxygen tension during the first 20 to 30 minutes amounting to 1 mm.Hg per minute. The fall in oxygen tension over a further period of 30 minutes was much slower, amounting to only 2 to 3 mm.Hg. The overall decline in the oxygen tension of the blood samples amounted to 12% of the initial level.

Other samples of arterial blood contained in capped syringes were plunged into iced water immediately after collection. Aliquots were withdrawn, under anaerobic conditions, into capillary tubes at selected times and the oxygen tension determined. As oxygen is continuously reduced at the platinum cathode rewarming cannot be carried out in the oxygen cell, therefore all samples were prewarmed before they were introduced into the oxygen cell.

Oxygen tension measurements made at 38°C on three
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**Figure 4** The fall in the $P_{O_2}$ level in three samples of blood when stored anaerobically at room temperature ($22^\circ$C). Measurements were made at $38^\circ$C. The broken line represents the tension levels extrapolated to zero time.

**Figure 5** The graph shows the stability of $P_{O_2}$ in three different blood samples stored anaerobically at $4^\circ$C in glass syringes. Aliquots were withdrawn and quickly rewarmed anaerobically to $38^\circ$C before the $P_{O_2}$ was measured.

different samples of blood, kept anaerobically in iced water, over a period of 60 minutes are shown in Figure 5. The sample of blood with the initial oxygen tension of 200 mm.Hg showed a steady fall in tension after 20 minutes, and also for the remainder of the time observed. No alteration in tension was measured in the blood with the initial tension of 130 mm.Hg. A fall of 2 mm.Hg in the oxygen tension measurement at 25 minutes in the blood sample of 85 mm.Hg tension was detected. However, measurements during the next 30 minutes showed no further variations.

It can be concluded from these observations that the technique of rapid anaerobic cooling of blood samples to the temperature of iced water and the rewarming to $38^\circ$C before making the oxygen tension measurement is a satisfactory method of maintaining the oxygen tension of a specimen of blood.

**Comparison of $P_{O_2}$ levels in arterial blood and 'arterialized' capillary blood** The risks and discomfort of frequent arterial punctures or that from having an indwelling arterial canula in place over a period of days is far from desirable. However, the oxygen requirements of certain types of patients suffering from severe respiratory diseases are such that frequent monitoring of the blood oxygen tension is becoming of prime importance (Hutchison, Flenley, and Donald, 1964; Campbell, 1964).

The practical advantages of acid-base determination on arterialized capillary blood are well established. Siggaard Andersen et al. (1962) have established the agreement between arterial and capillary blood oxygen saturation levels. The relationship between the oxygen tension of arterial blood and capillary blood was studied, particularly with regard to methods of collecting the capillary specimen.

Arterial blood was obtained from patients who were having arterial cannulation for clinical investigations. Blood was collected in heparinized glass syringes and capped immediately.

'Arterialized' capillary blood was taken from an ear puncture into heparinised capillary tubes at the identical time of obtaining the arterial blood sample. The capillary tubes were sealed with plasticine and placed in a polythene envelope. If oxygen tension measurements could not be made immediately, the capped syringe and the polythene envelope were placed in ice-cold water and transported to the laboratory. Oxygen tension measurements were made after the samples were rewarmed to $38^\circ$C.

The relationship of arterial blood oxygen tensions to the oxygen tension measured on the blood from the capillary is shown in Figure 6.

The regression coefficient: $b = 0.9277$ with 95% confidence limits for $b$ as 1.0236 and 0.8318.

Two possible sources of error in capillary collection were pressure on the ear tissue causing mixing of venous blood with arterial blood and exposure of the blood droplet to atmospheric oxygen. If excess pressure was required to obtain a blood specimen it invariably gave low results. The effect of exposure of blood of varying oxygen tensions to atmospheric oxygen at $38^\circ$C was studied as follows. Small volumes of blood of a known oxygen tension kept anaerobically in a syringe at $38^\circ$C were...
exposed on the back of a hand to air for varying lengths of time. A control oxygen tension value on the blood in the syringe was estimated at suitable intervals. The results are shown in Fig. 7, and demonstrate the loss of oxygen tension in blood specimens where the tension is in excess of atmospheric oxygen tension (142 mm.Hg), and the gain in blood oxygen tension in those specimens where the initial oxygen tension was lower than that of atmospheric oxygen tension. Because of the practical difficulties involved, the exposure times were in excess of the actual exposure times experienced in the taking of the capillary samples.

**DISCUSSION**

Recently the assessment of arterial oxygen tension in patients in respiratory distress has been strongly advocated by Hutchison et al. (1964) and by Campbell (1964) as a means of achieving a more efficient control of the level of blood Pco₂. In other clinical conditions such as those involving hypothermia and extracorporeal circulation the frequent assessment of Pao₂ can be used as an early indication of the development of inadequate oxygenation and the accompanying metabolic acidosis.

The development of the solid platinum oxygen electrode protected by a hydrophobic membrane from the contaminating fluids by Clark (1956) has proved an efficient and accurate system for the assessment of blood oxygen tension. The absence of such a commercial electrode system has, however, in the past limited oxygen tension measurements to large departments which have specialized in certain aspects of respiratory function, while the routine investigation of blood oxygenation has been largely based on the measurement of the percentage oxygen saturation of blood. However, recent investigations by Naeraa (1964) have illustrated the limitations of this particular measurement, especially if the percentage oxygen saturation is used to calculate the oxygen tension of the blood. In a large number of clinical states the partial pressure of the blood oxygen is a more important parameter than the percentage saturation of the blood. At tissue level the factor which controls the rate of movement of the oxygen into the tissues is the tension of the gas and not the degree of saturation of the blood.

The electrode system described here is an integral component of pH equipment which is currently in use in a large number of hospital laboratories, and therefore requires no further great expenditure to measure the actual oxygen tension of blood.

Compared with other commercially available electrode systems this system requires a relatively small volume of blood. This has been made possible by the use of a very thin polypropylene membrane which is less permeable to oxygen than to the teflon
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used in the early electrode systems, and by the use of a smaller surface area of platinum electrode.

The combination of these two factors is sufficient to reduce the oxygen consumption in the vicinity of the membrane to a point where breakdown of the oxygen gradients by stirring is no longer necessary.

In conditions where serial determinations are necessary in the long-term monitoring of patients and when an indwelling arterial catheter is not present, the availability of a method which only requires a small sample of blood obtainable by a simple technique is of considerable importance. The use of the volume reduction inserts in the inlet and outlet blood parts of the oxygen cell allow the measurement of Po2 to be made accurately on a volume which can be readily obtained by capillary sampling. Provided the arterIALIZATION of the capillary blood is possible, and this is more easily achieved in the ear rather than the finger, the agreement between such arterIALIZED capillary blood and arterial blood is sufficiently accurate for most clinical purposes. If a state of shock exists, the arterIALIZATION of the capillary blood is difficult and may be impossible, and arterial puncture should be performed.

In this method a strictly controlled procedure is essential. If blood is taken by a syringe it should be of glass rather than plastic, as the latter absorbs oxygen. In the event of a delay between taking the blood and measuring the oxygen tension, steps must be taken to prevent a fall in oxygen tension. The use of enzyme poisons such as fluoride (Torres, 1963) and cyanide (Asmussen and Nielsen, 1961) have proved unsuccessful as a means of stabilizing the oxygen tension. However, the simple technique of rapidly lowering the temperature of the blood to 4°C did succeed in maintaining a stable Po2 for a period of 60 minutes. Of equal importance is the rapid anaerobic rewarming of the blood in the syringe or capillary to the temperature of measurement. If the blood is allowed to warm in the measuring chamber, results will be low because of the continual reduction of oxygen at the cathode of the oxygen electrode. The precision and stability of the electrode system is well illustrated by the standard deviations recorded on the repetitive analyses of blood samples at the three different oxygen tensions and by the excellent agreement of Po2 values obtained on the duplicate samples measured simultaneously on the established Bishop type oxygen electrode and the Radiometer system. The degree of stability of the electrode system was found to be largely dependent on the state of the membrane, and when correctly applied and maintained, the stability remained constant for the expected working life.

It was observed that in a large number of the patients the blood Po2 was considerably lower than that expected from the composition of the anaesthetic/oxygen gas mixture which was in use during the artificial ventilation. Nunn (1964) has reported a similar observation in patients breathing spontaneously and suggests that in order to maintain a normal blood Po2 an alveolar P02 as high as 200 mm.Hg, or an inspired oxygen concentration of 35% is required in patients being artificially ventilated.

I wish to express my thanks to Mrs. A. Holyoak, Department of Medicine, Birmingham University, for Po2 measurements on the Bishop electrode system; to Dr. Clark and staff of the X-ray Department, Queen Elizabeth Hospital, for their cooperation during part of the investigation, and to Radiometer, Copenhagen, who kindly supplied the Po2 electrode equipment.

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