BLOOD OXYGEN AND CARBON DIOXIDE TRANSPORT IN MAN.

by

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UNITS

The units used in this thesis were selected following the guidelines laid down by the editorial board of the journal Clinical Science who recommend the use of S.I. units for all values excepting gas tensions, where, at the author's discretion, values may be expressed as mmHg or kPa, with the appropriate factors for conversion from one to the other.

In this thesis gas tensions are given as mmHg, and may be converted to kPa by:

$$0.133\text{mmHg} = \text{kPa}$$

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Abstract

The effect of long term domiciliary oxygen therapy on the position and shape of the oxygen dissociation curve, together with other haematologic variables such as 2,3-diphosphoglycerate (2,3-DPG), haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin concentration, and arterial blood gas and pH values, has been studied in patients with chronic bronchitis. Twenty-six patients were randomly allocated to receive either no oxygen therapy or 15 hours per day oxygen therapy. The two groups were initially well matched for age and disablement.

Haemoglobin concentration and packed cell volume (P.C.V.) were the only variables to differ significantly between the two groups of patients after start of oxygen treatment, but those patients receiving oxygen therapy showed significant decreases in 2,3-DPG, together with haemoglobin concentration and P.C.V. from the pre-treatment values.

The effect of elevated COHb levels, (similar to those encountered in patients who smoke) on exercise tolerance was studied in patients with chronic bronchitis, whose exercise capacity was already limited due to hypoxaemia. Exercise tolerance, assessed by the distance walked in 12 minutes, was lower after elevation of COHb levels, and a significant correlation was established between calculated femoral venous oxygen tensions (assuming $\Delta(A-V)O_2$ values of 7 and 10 ml/100 ml blood) and the 12-minute walking distance. ($r=0.34$, $P<0.01$ and $r=0.33$, $P<0.01$)

The influence of cigarette smoking on the genesis of secondary polycythaemia in patients with chronic bronchitis was also investigated. Those patients who smoked had an increased erythropoietic response to hypoxaemia. A negative relationship was demonstrated between arterial oxygen saturation and red cell mass, if the prevailing carboxyhaemoglobin (COHb) level in each patient was taken into account when determining arterial oxygen saturation ($r=-0.56$, $P<0.005$). A significant negative correlation was also established between the calculated venous oxygen tension of the renal medulla and red cell mass ($r=-0.51$, $P<0.001$).

The magnitude of the Christiansen, Douglas, Haldane (CDH) effect was determined in normal men and women, and also before and after COHb elevation in patients with chronic bronchitis. It was found to be similar in all groups and was not affected by changes in COHb levels. Using the CDH effect determined in these patients, the contribution of this effect towards the rise in PCO_2 seen in hypercapnic bronchitic subjects when given oxygen was determined, and the importance of this effect is discussed.

The magnitude of the CDH effect remained constant through the menstrual cycle in normal women, but in women taking a combined oestrogen/progesterone oral contraceptive (ethinyloestradiol 30μg, D-norgestrel 0.15 mg), the CDH effect at menstruation was significantly lower ($P<0.001$) than that in normal women, but there was no difference at mid cycle. The reason for this difference is not clear.

CHAPTER 1

Historical Introduction

The quest for knowledge about science, and in particular respiration, dates back to ancient times. Empedocles in the fifth century B.C. was the first to formulate a theory of the ebbing and flowing of blood to and from the heart, but he believed respiration took place through the entire skin.

The great Hippocratic era gave many classic descriptions of thoracic disease, as well as the theory that the heart is the seat of innate heat, and the purpose of respiration is to cool this fiery process. However, the views of one man, Galen (130 - 199 A.D.), dominated the study of respiration for a longer span than those of anyone else, and although some of these views were totally unsupported by experiment, they were believed and taught until the 17th century.

In the early 17th century Harvey described the observations leading to his theory of the circulation of blood, but this momentous discovery threw no positive light on the physiology of breathing, and it was still generally believed that the main function of respiration was to cool the blood.

Further progress in physiology required corresponding progress in chemistry, a requirement met before the end of the 17th century by Boyle, Hook, Lower and Mayow. Boyle and Hook demonstrated that animals and fire would not survive in a vacuum and Mayow demonstrated a decrease in the volume of air accompanying respiration and combustion, suggesting a similarity between the two processes. Lower was the first to notice the colour change occurring when venous blood becomes arterial as a result of fresh air in the lungs.

Following the work of these chemists, the understanding of respiration lapsed for over a century, mainly due to the erroneous phlogiston theory of Georg Ernst Stahl, and the next important step in the understanding of respiration came in the middle of the 18th century when Joseph Black discovered 'fixed air' or carbon dioxide was liberated from carbonates by acid, and was also given off by the lungs. Although this discovery should have heralded the end of the phlogiston theory, it was too deeply established to be removed at the first attempt, and, in fact, when Priestly discovered oxygen shortly after this, he interpreted his findings as supporting the phlogiston theory. Because of this, many historians feel that oxygen was merely isolated by Priestly, and that Lavoisier really discovered it, by showing that in combustion, oxygen combined with carbon to form carbon dioxide, whilst hydrogen combined with oxygen to form water, and that oxygen was, in fact, essential for the maintenance of life.

Haemoglobin

Iron was known to be a constituent of blood as early as 1747, when Menghini separated it from the ash of blood by a magnet, and the presence of iron was proved chemically in 1825 by Endelhardt. Hoppe-Seyler showed in 1864 that haemoglobin combines loosely with oxygen, but it was not until 1938 that Haurowitz discovered the reversible change of structure which accompanies the combination of haemoglobin with oxygen.

Much work was carried out around this time on a wide range of proteins, including haemoglobin, and almost complete knowledge was gained as to the number and nature of the amino acid residues present in the molecule. However, it was not until the 1950's, following the work of Sanger on the structure of insulin, which prompted similar studies on other proteins, that the exact chemical sequence of haemoglobin was discovered.

It was Perutz and co-workers who, in 1960, published work describing the architecture of the haemoglobin molecule in outline. This early study, using horse oxyhaemoglobin, revealed nothing of the inter-relationship within the amino acid sequence, but did show the four separate globin chains which are identical in pairs, the members of one pair known as alpha chains and those of the other as beta chains. As haemoglobin was known to be similar to myoglobin, the structure of which was known (Kendrew and coworkers, 1960), Perutz was able to interpret the results of his work further than would have been possible on the basis of the results alone, and inferred that there were in each chain a large proportion of alpha-helices. Seven segments of alpha-helices were found in the alpha chains (denoted A,B,C,E,F,G,H), and eight in the beta chains (denoted A,B,C,D,E,F,G,H). The

haem groups were seen to lie in separate pockets on the surface of the molecule (Fig. 1). Each haem group contains a porphyrin ring and a ferrous ion which can form fivefold or sixfold co-ordination complexes. Four of the co-ordinated groups are the nitrogen atoms of the porphyrin rings and the fifth is an imidazole group of the proximal histidine residue in the globin chain. When present, the sixth co-ordinate is oxygen and, because the surrounds of the haem groups are strongly hydrophobic, oxygen can combine without oxidising the ferrous ion to the ferric form (Perutz, 1964). The detailed sequences of the alpha and beta chains of normal human haemoglobin were determined by Braunitzer and co-workers and Konigsberg and co-workers in 1961. The alpha chains are made up of 141 amino acid residues each, and the beta chains of 146 residues each.

Having established the structure of oxyhaemoglobin, Perutz and his co-workers turned their attention to the structure of deoxyhaemoglobin. In 1967 Muirhead, Cox, Mazzarella and Perutz compared electron density maps of oxy- and deoxyhaemoglobin at a relatively low resolution and were unable to find any significant differences in the folding or tertiary structure of the molecules, but the results showed differences in the quaternary structures, i.e. the positions of the subunits. It was shown that the two beta chains are displaced in the oxy-haemoglobin molecule, which reduces the entrance to the central cavity of the molecule. Also, there is movement of the alpha chains relative to each other. Although this finding had great implications for the understanding of the function of haemoglobin, it was not until higher resolution techniques were used by Perutz, Muirhead, Cox and Goaman in 1968 and Bolton and Perutz

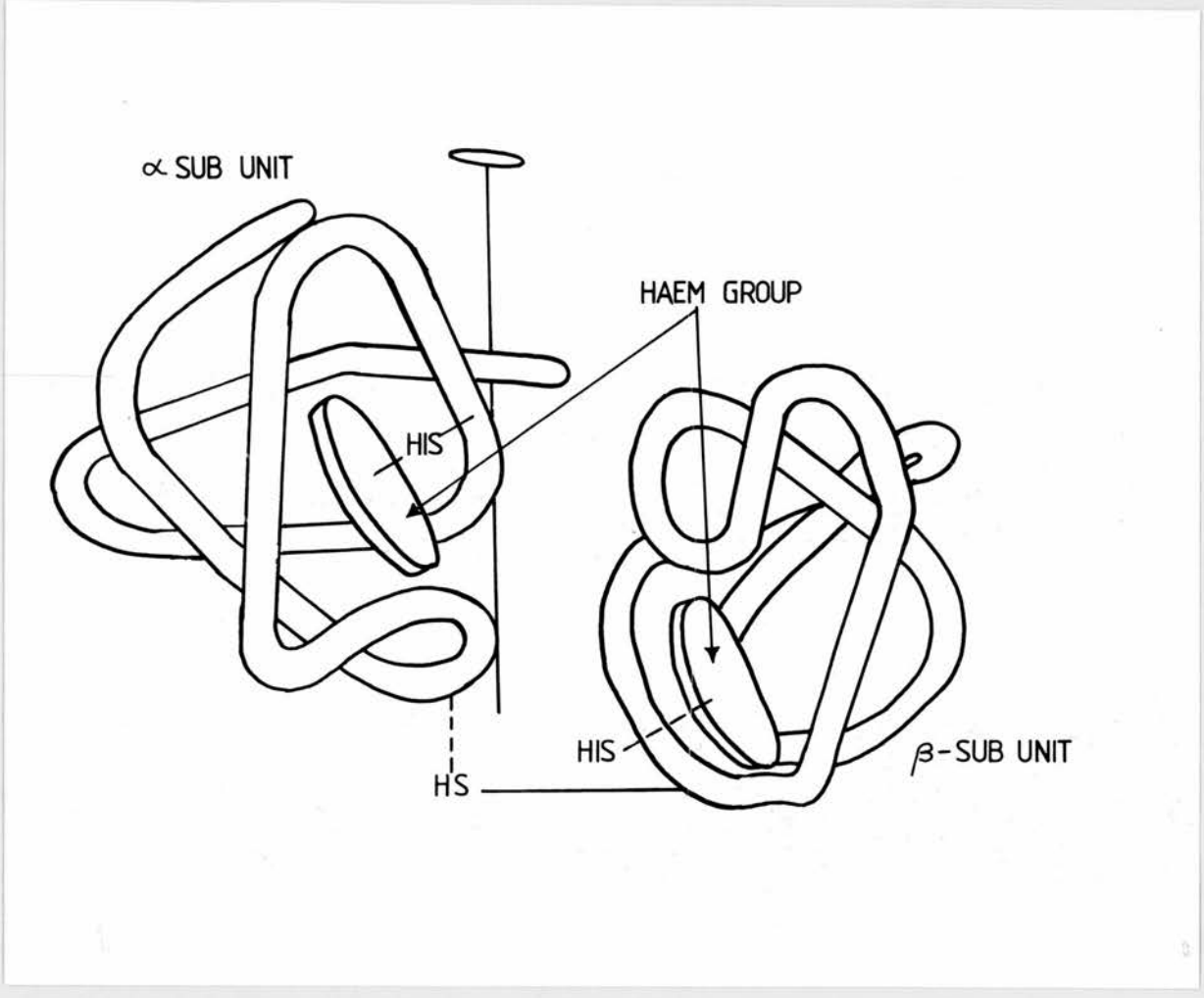


Figure 1

The chain configuration of the ∝ and β subunits of haemoglobin, showing the position of the haem groups in each subunit. (Perutz, Rossmann, Cullis, Muirhead, Will and North, 1960).

in 1970 that the important differences between the tertiary structures were found. From this higher resolution work, numerous hydrogen bonds were found between the alpha 1 - beta 1 and alpha 2 - beta 2 subunits, which were so strong that in the transition between the oxy- and deoxy- structures they moved as rigid bodies. On the other hand, the contact alpha 1 - beta 2 in the oxy-structure was found to be quite different from that in the deoxy-structure. Salt bridges between the last amino acid residues of each subunit, which were present in the deoxyhaemoglobin molecule, were absent in the oxy-structure. These findings, together with the movement of the iron atom out of the plane of the porphyrin ring in the deoxy-molecule, provided the basis for a model to describe the co-operative effect of the binding of oxygen to haemoglobin, that is, the way in which the binding of one oxygen molecule facilitates the binding of the next one. This co-operative effect was first observed by Bohr in 1903 and accounts for the sigmoid shape of the oxygen dissociation curve.

A general model of co-operativity had already been applied to describe facilitated binding of ligand to a protein provoked by the preceeding binding of the same ligand, by Monod, Wyman and Changeux in 1965. They postulated that if there are only two structural conformations of a protein, the one with fewer bonds would be able to develop greater activity, whereas with stronger bonds between the subunits, the activity would be reduced. They termed the structure with fewer bonds R for relaxed and the other structure T for tense. This theory simplified the interpretation of co-operative effects enormously, but it was Perutz who, in 1970, proposed a model to describe co-operativity,

or haem-haem interaction within the haemoglobin molecule, based on the existence of two alternative quaternary structures (Perutz, 1970).

In Perutz' theory the deoxyhaemoglobin molecule represents the T state and the oxyhaemoglobin molecule the R state of the Monod, Wyman, Changeux model. Previous work carried out by Hoard (1968) had shown that in the deoxy-conformation, or T state, the ferrous ion is in a high spin state with a wide diameter, and thus protrudes out of the plane of the porphyrin ring, but in the oxy-conformation or R state, it is small enough to fit into the porphyrin ring. Perutz proposed that as oxygen binds, the distance between the iron ion and the porphyrin ring decreases sequentially, and the ion carries the proximal histidine and the F helix of the globin chain with it, causing a pocket in the molecule between helices F and H to narrow, expelling a tyrosine side chain. This movement, in turn, ruptures a salt bridge between the two subunits, liberating Bohr protons. As each salt bridge is broken, more constraints holding the molecule in the deoxy conformation are removed, and the equilibrium between the two conformations is tipped towards the oxy-conformation, increasing the affinity of haemoglobin for subsequent oxygen molecules.

Much of this theory was conjecture, and the structural connection between the movements of the iron ions and those of the carboxy termini could not be described. Later, results of Anderson (1973 and 1975) suggested that such gross movements did not occur.

Maps are now available at resolutions of 2.5 $\mathring{A}$ for human deoxyhaemoglobin and 2.0 $\mathring{A}$ for horse methaemoglobin,

and more detailed comparisons of the two structures have been achieved (Fermi, 1975; Ladner, Heidner and Perutz, 1977). These more recent results show many qualitative similarities to earlier results, but differ in quantitative aspects, which provide a more accurate picture of certain important changes in the molecule, and cast doubts on Perutz' theory that the cause of the low affinity of the deoxyhaemoglobin conformation was the energy required to break the salt bridges.

An alternative theory was proposed by Baldwin and Chothia (1979). As the iron binds oxygen, its position of lowest energy is in the plane of the porphyrin ring, but steric repulsions between the iron and the F helix prevent movement of the iron into the ring. Because the molecule is closely packed, the F helix cannot move to relax the strain, and decreases the stability of the quaternary structure so that the proportion of molecules in the oxy-conformation increases as successive oxygen molecules bind. The change in the quaternary structure induces tertiary structure changes that reposition the F helix relative to the haem group removing the steric repulsion, and facilitating the binding of oxygen.

Transport of oxygen by the blood

It was known in 1669 that venous blood turns red when exposed to air, but further knowledge of the transport functions of the blood was not forthcoming until the early 19th century when Gustav Magnus established that arterial blood contained more oxygen than venous blood, but less carbonic acid. Hoppe-Seyler, one of the pioneering workers on haemoglobin in the 19th century, attributed the oxygen combining properties of blood to haemoglobin, and a chemical combination was suggested by Claude Bernard's results in 1858 which showed that carbon monoxide displaced oxygen from the blood.

Paul Bert was the first to investigate the oxygen dissociation curve in 1872 by plotting the oxygen content of blood against the barometric pressure of air, but no further work on the dissociation curve was carried out until 1886, when Christian Bohr plotted the oxygen dissociation curve of purified haemoglobin, which resembled a hyperbola as there were no values below 30% oxygen saturation. Next, Hüfner, who had studied haemoglobin for many years, showed that the dissociation curve could be fitted by a rectangular hyperbola, as he had assumed that the reaction of haemoglobin with oxygen was unimolecular. However, in 1903, Bohr disproved this assumption by plotting the whole oxygen dissociation curve, and showing it to have a sigmoid form.

Many workers have investigated the oxygen dissociation curve, both mathematically and experimentally, since Bohr revealed the sigmoid shape. In 1910 A.V. Hill attempted a mathematical analysis of the curve. He assumed that haemoglobin molecules

in solution aggregate in the presence of salts, with 'n' molecules of haemoglobin in the aggregate, while in distilled water, haemoglobin would be monomeric. The theory is formulated in the Hill equation:-

$$y = k\,(pO_2)^n \,/\, [1 - (pO_2)^n]$$ where k and n are constants

y = fractional saturation of haemoglobin with oxygen

The slope of the "Hill plot", obtained by plotting log (y/1 - y) against log pO_2, can be used as an indicator of the degree of co-operativity or haem-haem interaction. If there is no haem-haem interaction the slope or 'n' is unity. The 'n' value of oxygen binding to haemoglobin under physiological conditions is almost constant over a saturation range of 20 - 80% and has a value of 2.6 - 2.8.

Barcroft tested the validity of Hill's equation by comparing oxygen dissociation curves calculated using the formula, with those determined experimentally, and found very good agreement, both for human blood and that of sheep, dog and cat. Thus, for many years studies on oxygen and haemoglobin combination were based on the dissociation curve of blood as presented by Barcroft in 1914.

However, in 1924, Bock, Field and Adair were unable to construct oxygen dissociation curves which verified Barcroft's curves, and although they found Hill's formula was not adequate to describe the whole curve, it was sufficiently accurate over most of the physiological range, and it is still widely used as it provides an informative and convenient way of representing the oxygen dissociation curve.

In 1925 Adair formulated his general intermediate compound hypothesis. He proposed that the oxyhaemoglobin reaction takes place in four steps, each one having its own microscopic equilibrium constant. As application of this theory requires accurate measurement at the top and bottom of the curve, it was not until 1972 that data was fitted successfully to the Adair equation (Roughton, DeLand, Kernohan and Severinghaus).

Since 1925 several attempts have been made by various workers to reduce the number of Adair constants, notably the theories of Pauling (1935), Monod, Wyman and Changeux (1965) and Koshland (1966), but Roughton rejected these models on the basis of very accurate measurements of the dissociation curve.

The oxygen dissociation curve has been investigated experimentally in great detail by many workers, and a range of results regarding the position and shape of the curve in human subjects has emerged. However, in contrast to the variable position of the curve in health and disease, its shape is widely believed to be constant between oxygen saturations of 20 - 80% at low carboxyhaemoglobin levels. It has, therefore, been accepted that the P50, which is the oxygen tension required to produce 50% saturation of haemoglobin with oxygen (pH 7.4, PCO_2 40 mm Hg, temperature 37^{o}C), is sufficient to characterise the position of the curve.

In 1966, Severinghaus standardised the oxygen dissociation curve by collecting results from many sources and constructing an average curve. The P50 of this curve is 26.6 mm Hg at pH 7.4, PCO_2 40 mm Hg and base excess 0, with a value for Hill's 'n' of 2.6.

An increase in P50 indicates a decrease in haemoglobin oxygen affinity, and a decrease in P50, an increase in haemoglobin

oxygen affinity, and once the shape of the oxygen dissociation curve had been established, many workers turned their attention to investigating the factors which alter the P50 and control the oxygen affinity of haemoglobin.

(i) Effect of carbon dioxide

In 1904 an important discovery was made by Bohr, Hasselbalch and Krogh, namely that CO_2, when added to blood, expels oxygen, a phenomenon which became known as the Bohr effect. Although Bohr suggested that the converse effect may exist, i.e. that oxygen would drive out CO_2 from the blood, they could not demonstrate this at the time, and, in fact, wrote "no certain action of oxygen pressure on the simultaneous absorption of CO_2 was observed; if any such action exists, it is at any rate small and can have no special significance for the conditions in the living body".

In 1910 Barcroft proved that the expulsion of oxygen from the blood was not a property of carbonic acid alone. He found that lactic acid caused a rightward shift in the dissociation curve and pointed out the usefulness of this mechanism, as during oxygen shortage, lactic acid is produced which, by reducing the affinity of haemoglobin for oxygen, will facilitate oxygen release to the tissues. The Bohr effect has thus come to mean the effect of changing pH on the oxygen dissociation curve, as opposed to the effect of changing CO_2 concentration, and is expressed as $\Delta \log PO_2/\Delta pH$.

Ten years after the discovery by Bohr, Christiansen, Douglas and Haldane demonstrated the reciprocal effect, i.e. that oxygenation of blood caused a decrease in total CO_2 content,

a phenomenon which they proposed may be due to oxyhaemoglobin being a stronger acid than reduced haemoglobin and thereby driving CO_2 out of solution. Henderson explained this by proposing that the combination of haemoglobin with oxygen increases the dissociation of a single monovalent acid group in haemoglobin and this would lead to the release of protons on oxygenation (Henderson, 1920).

This proposal could not be tested experimentally at this time as it required accurate determination of pH and this was not possible before 1937. However, with the development of the glass electrode, German and Wyman were able to measure titration curves of oxy- and deoxy-haemoglobin with greater accuracy. They found that oxyhaemoglobin was indeed a stronger acid than reduced haemoglobin over the pH range 6.1 - 8.9, but the reverse is true between 6.1 - 4.5. At a pH of 7.0 they found that the change in the charge on the haemoglobin molecule as it becomes oxygenated is -0.5 m equivalent H^+ per m equivalent haemoglobin, the experiment being performed on haemoglobin solution in the absence of CO_2. This oxygen-linked hydrogen ion binding, first suggested by the work of Christiansen, Douglas and Haldane has become known as the Haldane effect and is expressed as (Δ Hb-bound H^+) / (Δ HbO_2 from 1 - 100%).

In 1948 Wyman, using the linked function principle, showed that the Haldane coefficient and the Bohr coefficient should be equal and this theory was given credence when Dill, Graybiel, Hurtado, Taquini, in 1940, produced a value of -0.48 for the Bohr coefficient which compared favourably with the value of -0.50 for the Haldane coefficient given by German and Wyman (1937).

However, when investigating the Bohr effect, the pH of the blood can be altered by two methods: the addition of CO_2 or the addition of fixed acid or base. The difference between these two methods only became significant when, in 1955, Margaria and Milla compared the effect of respiratory (i.e. CO_2-induced) pH changes and metabolic (i.e. fixed acid induced) pH changes on the oxygen dissociation curve. They found that at any given pH, a pure solution of horse haemoglobin, in the absence of CO_2, has a greater oxygen affinity than a haemoglobin solution in the presence of CO_2, confirming earlier work of Margaria and Green (1933). They attributed this effect to the formation of CO_2-haemoglobin complex in the form of a carbamate, which has a lower affinity for oxygen than simple haemoglobin, and whose formation has been shown to be proportional to pH (Ferguson and Roughton, 1934[2]).

This additional effect of CO_2 on the dissociation curve was later confirmed in human whole blood by Naeraa, Strange Petersen and Boye (1963), who, on the basis of their results, proposed that the Bohr factor would alter depending on whether the pH was changed by CO_2 or fixed acid. However, much controversy arose when Colman and Longmuir (1963) and Astrup, Engel, Severinghaus and Munson (1965) produced results which suggested there was no pH-independent effect of CO_2, but the issue was settled in 1966 when Naeraa, Strange Petersen, Boye and Severinghaus found the CO_2 Bohr effect approximates $\Delta \log PO_2 = -0.5\ \Delta pH$, whereas the fixed acid Bohr effect, measured at constant PCO_2, approximates $\Delta \log PO_2 = -0.4\ \Delta pH$, the difference being due to the formation of carbamate, which accounts for 20% of the CO_2 Bohr effect.

In 1969, Bauer investigated the fixed acid Bohr effect in the presence and absence of CO_2, using purified haemoglobin solutions. He found the fixed acid Bohr effect, in the absence of CO_2, was similar to the CO_2 Bohr effect, but in the presence of CO_2, the fixed acid Bohr effect was reduced. This was confirmed by Dahms, Horvath, Luzzana, Rossi-Bernardi, Roughton and Stella (1972) and also by Arturson, Garby, Robert and Zaar (1974) who used whole blood, and is due to the pH-dependent formation of carbamates when CO_2 is present, for at low pH there is little carbamate formation, but as the pH rises, carbamate formation increases and although the increased pH acts to lower the P50, the increased carbamate counters this, with the result that $\Delta \log P50/\Delta pH$ is lower than in the absence of CO_2, where no carbamates form on raising pH, and thus no antagonism of the Bohr effect is seen.

The effect of CO_2 on the Haldane coefficient was first demonstrated by Rossi-Bernardi and Roughton (1967). They found that the Haldane effect in the absence of CO_2 was constant over the pH range 6.8 - 7.6. However, when PCO_2 = 39 mm Hg, the Haldane effect was halved at pH 7.2, and decreased further as the pH increased. In fact, at PCO_2 50 mm Hg and pH 7.5, the Haldane effect was reversed and haemoglobin solutions became more alkaline on oxygenation. They explained this as being due to the buffering effect of carbamate. As carbamino-bound CO_2 dissociates from haemoglobin on oxygenation, it removes H^+ ions from solution, thereby reducing the change in H^+ ions caused by oxygenation. As carbamate formation increases at high pH and PCO_2 values, a point is reached where as many protons are removed by dissociation of carbamate as are added by oxygenation,

and beyond this point the Haldane effect will be reversed. This reduction of the Haldane effect by CO_2 has been confirmed in haemoglobin solutions by Kilmartin and Rossi-Bernardi (1969) and Siggaard-Anderson (1971) and in whole blood by Siggaard-Anderson and Salling (1971). However, Mithoefer, Thibeault and Bossman (1969) were unable to show the reversal of the Haldane effect in dog whole blood despite the use of higher PCO_2 and pH values than Rossi-Bernardi and Roughton (1967).

According to Wyman (1948) the Bohr effect is determined exclusively by the interaction between oxygen and hydrogen ions, and not influenced by other ligands of haemoglobin. However, it is now known that the Bohr and Haldane effects vary, not only with CO_2 concentration, but also with 2,3-diphosphoglycerate (2,3-DPG) concentration and oxygen saturation.

The effect of 2,3-DPG on the fixed acid Bohr effect in haemoglobin solutions was first investigated by Bauer (1969), who found little effect of 2,3-DPG in the absence of CO_2, but at PCO_2 levels of 40 mm Hg, 2,3-DPG caused an increase in the Bohr effect. As 2,3-DPG influenced the Bohr effect only in the presence of CO_2, Bauer proposed that it acts by reducing carbamate formation at increased pH, and in 1970 was able to prove this. Subsequent work on whole blood confirmed the effect of 2,3-DPG on the fixed acid Bohr effect (Wranne, Woodson, Detter, 1972; Hlastala and Woodson, 1975; Duhm, 1976), but the CO_2 Bohr effect was reduced in the presence of 2,3-DPG (Wranne, Woodson and Detter, 1972). This again is due to suppression of carbamate formation by 2,3-DPG. As carbamate formation at high pH is minimal under these conditions due to the necessarily low PCO_2, it is not affected significantly by 2,3-DPG, but as the pH is lowered by an

increased PCO_2, 2,3-DPG depresses carbamate formation, thereby reducing the rightward shift of the oxygen dissociation curve and hence the CO_2 Bohr effect. Investigation of these complex inter-reactions led Hlastala and Woodson (1975) to the conclusion that at normal levels of 2,3-DPG, the CO_2 Bohr effect is primarily a direct effect of H^+ ions, but on 2,3-DPG depletion, carbamate formation plays a much more important role.

The Haldane effect was also found to be enhanced by 2,3-DPG (Siggaard-Anderson, 1971), a result which bears out the proposal of Arnone (1972), that the binding of 2,3-DPG is accompanied by an increase in the pK value of the positive groups in haemoglobin. This enhancement of the Haldane effect by 2,3-DPG may explain why Mithoefer et al (1969) were unable to demonstrate the reversal of the Haldane effect by CO_2 when using fresh whole blood, and in fact, Bauer (1970) suggested that if 2,3-DPG had been present in the haemoglobin solutions used by Rossi-Bernardi and Roughton (1967), the Haldane effect would not have been reversed.

Until the mid-1960's it was generally believed that the Bohr effect was independent of oxygen saturation (Antonini, Wyman, Brunori, Bacci, Fronticelli and Rossi-Fanelli, 1963), i.e. the Bohr effect at 50% oxygen saturation was indicative of that at all other saturations, but in 1967 Kernohan and Forster showed that at high oxygen saturations, the Bohr effect was lower than at mid saturation values.

In 1972, Garby, Robert and Zaar investigated the CO_2 and fixed acid Bohr effects over the whole oxygen saturation range, and found they did indeed change with oxygen saturation. The fixed acid Bohr effect was maximum at mid saturation values and fell off as saturations approached 0 and 100%, whereas the CO_2 Bohr effect was maximum at low oxygen saturation values and became

lower as saturation increased, reflecting the reduction of carbamate formation as the oxygen saturation rises.

The binding sites of the oxygen-linked hydrogen ions have now been mapped as a result of an extensive study involving x-ray crystallography and chemical modification of the haemoglobin molecule. From the work of Benesch and Benesch (1961) and Perutz (1970) it appears that the C-terminal histidines of the β-chains of haemoglobin account for 50% of the Bohr protons released on oxygenation, and Kilmartin and Rossi-Bernard in 1969 showed that the amino group of the N-terminal valine of the two α-chains accounts for a further 25% of the Bohr effect. Perutz in 1970 also suggested that the imino group of one of the histidine residues of the α-chains may contribute to the Bohr protons. According to a theory by Perutz, all the above groups participate in formation of salt bridges in the deoxyhaemoglobin molecule, which causes the pK value for the dissociation of protons to be raised. In oxyhaemoglobin, the salt bridges rupture, the pK values fall and protons are released. As the pH becomes more acid, the weak bases in the haemoglobin molecule (with pK values at about neutrality) will be charged and able to form salt bridges, thereby increasing the stability of the deoxy form relative to the oxy form and lowering the oxygen affinity. As the pH becomes more alkaline, the weak bases will be uncharged, so that salt bridges will not be formed, thus decreasing the stability of the deoxy form and increasing the oxygen affinity.

The additional effect of CO_2 on oxygen affinity is also believed to be due to salt bridge formation between the negatively charged carbamino group and a positively charged group in the deoxy-haemoglobin molecules, probably the ε-amino group of lysine 82 in the β-chain. This salt bridge stabilizes the deoxy conformation thereby lowering oxygen affinity.

ii) 2,3-Diphosphoglycerate

Almost 60 years ago Adair, Barcroft and Bock (1921) demonstrated that oxygen dissociation curves determined using haemoglobin solutions differed markedly from curves determined using blood dialysed against distilled water, which contained intact, though swollen, red blood cells. This led the authors to speculate that there may be a third substance, other than hydrogen ions and salts, which was present in the red cells, and formed an "integral part of the oxygen-haemoglobin complex", and hence altered the oxygen dissociation curve. This speculation received support from the work of Hill and Wolvekamp in 1936, which showed that the dialysable material from laked erythrocytes decreased the oxygen affinity of haemoglobin, but they did not link this effect to the presence of 2,3-diphosphoglycerate (2,3-DPG), which had been reported to be present in high concentrations in porcine red blood cells by Greenwald in 1925. Rapoport and Guest (1941) reported high concentrations of organic phosphates in most other mammalian species, with exceptions such as ungulates. They found 2,3-DPG was the most abundant of the organic phosphates in the red cell, with a molar concentration in human erythrocytes nearly equal to that of haemoglobin, at about 5 mM.

Although it seems appropriate that the red cell derives some advantage from this high concentration of 2,3-DPG, which is four times greater than the concentration of ATP, no important function was attributed to it until over 40 years after its discovery.

In 1965 Chanutin and Curnish found a slow moving electrophoretic band appeared after phosphate compounds were

added to purified haemoglobin solutions, proving that 2,3-DPG can form complexes with haemoglobin, at least in solutions of low ionic strength. However, the break-through in 2,3-DPG research did not come until 1967 when Benesch and Benesch and also Chanutin and Curnish, in the same year, discovered that 2,3-DPG could alter the oxyhaemoglobin dissociation curve in a dramatic way. Using dialysed human haemoglobin solutions, Benesch and Benesch found haemoglobin-oxygen affinity was very high and there was little haem-haem interaction. On addition of 2,3-DPG in physiological concentrations, the haem-haem interaction increased and oxygen affinity decreased to normal values. They found further addition of 2,3-DPG had little effect on the curve and concluded that P50 and Hill's 'n' reached their maximum values at the concentration of 2,3-DPG found in the red cell.

This displacement of oxygen by 2,3-DPG strongly suggested that deoxyhaemoglobin had a greater affinity for 2,3-DPG than oxyhaemoglobin and in 1968 Benesch, Benesch and Yu showed that DPG bound only to deoxyhaemoglobin in dilute haemoglobin solutions. They claimed the reduction in oxygen affinity by 2,3-DPG could be accounted for solely by this preferential binding to deoxyhaemoglobin, which stabilises the deoxy-conformation. However, in 1977, Goodford, Norrington, Paterson and Wootton showed that 2,3-DPG not only stabilises the deoxy-conformation of haemoglobin relative to the oxy-conformation, but it also lowers the oxygen affinity of the deoxy-conformation, producing a two conformational, three affinity state system.

In 1969, Garby, Gerber and de Verdier investigated the binding of 2,3-DPG to haemoglobin under conditions similar to those prevailing in the erythrocyte, and found that 2,3-DPG was,

in fact, bound by both deoxyhaemoglobin and oxyhaemoglobin, the affinity for 2,3-DPG of the former being, under physiological conditions, about twice that of the latter, a result confirmed by Chanutin (1969) and Caldwell, Nagel and Jaffe (1971).

Benesch et al (1968) investigated the effect of a wide range of 2,3-DPG concentrations on the oxygen dissociation curve of dilute haemoglobin solutions, and from their results concluded that variations of organic phosphate levels in the physiological range would have little effect on haemoglobin oxygen affinity. However, this was disputed in the same year by Lenfant, Torrance, English, Finch, Reynafarje, Ramos and Faura, and Rörth who found changes in 2,3-DPG levels corresponded to dramatic changes in oxygen-haemoglobin affinity in vivo. This anomaly prompted Duhm (1971) to investigate the differences in the effect of 2,3-DPG on haemoglobin in solution and in intact red cells. In the red cell he found a remarkable rightward shift in the dissociation curve when 2,3-DPG levels were increased above normal, and attributed this to a shift in the Donnan equilibrium induced by the accumulation of non-penetrating phosphate anions in the cell which causes a decrease in intracellular pH. This, in turn, lowers the oxygen affinity, by the Bohr effect, and accounts for the greater effect of 2,3-DPG on oxygen affinity of intact red cells. This effect was quantitated by Bellingham, Detter and Lenfant (1971) who found that a change in 2,3-DPG concentration of 6.0 μmoles/g haemoglobin alters the difference between plasma and red cell pH by 0.058 pH units, which accounts for about 35% of the overall change in $P50_{(7.4)}$ brought about by 2,3-DPG. Thus, in the red cell, 2,3-DPG influences oxygen affinity by two mechanisms: by a specific action on the haemoglobin molecule and also by an unspecific effect on red cell pH.

Benesch, Benesch and Yu (1969) found 2,3-DPG binding to haemoglobin was influenced by various environmental factors. pH was found to regulate 2,3-DPG binding, an increase in pH leading to a reduction in the binding of 2,3-DPG, an observation later confirmed by Garby and de Verdier (1971) and Caldwell, Nagel and Jaffe (1971). Consequently, the effect of 2,3-DPG on oxygen affinity is more pronounced at low pH values, and as a result of this, Benesch et al (1969) found 2,3-DPG enhanced the Bohr effect. After Siggaard Anderson (1971), De Bruin, Janssen and Van Os (1973) similarly showed 2,3-DPG increased the Haldane effect, expressed as the number of protons exchanged during oxygenation of deoxyhaemoglobin. They showed this enhancement was due to an uptake of protons following an increase in pK of the positive groups in the haemoglobin molecule, which occurs upon binding of 2,3-DPG to deoxyhaemoglobin.

Other factors which influence 2,3-DPG binding are: temperature, the binding constant decreasing as temperature increases, and anions such as chloride ions, which compete with 2,3-DPG for the oxygen-linked binding site. An apparent competition between 2,3-DPG and CO_2 was suggested by the work of Bauer (1969, 1970) which showed 2,3-DPG decreased the amount of carbamino-bound CO_2 at constant pH and PCO_2. This was confirmed by Tomita and Riggs (1971) who found CO_2 can totally abolish the effect of 2,3-DPG on oxygen affinity at high pH.

2,3-DPG Binding Sites

The important finding by Benesch et al (1968) of the simple 1 : 1 stoichiometry of the 2,3-DPG-haemoglobin interaction suggested that the 2,3-DPG binding site must lie on the dyad axis

of symmetry passing through the centre of the molecule. Subsequent work by Benesch, Benesch and Yu (1969) and Garby, Gerber and de Verdier (1969) showing that 2,3-DPG binding decreased considerably between pH 7.0 and 8.5, suggested that 2,3-DPG binds to positively charged groups with pK values in the physiological range of pH, and could therefore be accommodated by the α-amino groups or histidines.

It was shown by Benesch and Benesch (1969) that 2,3-DPG would bind to isolated β-chains, but not to isolated α-chains, suggesting that the binding of 2,3-DPG to normal haemoglobin occurs only in the β-chains.

Studies on human foetal haemoglobin by De Verdier and Garby (1969) produced additional information on the 2,3-DPG binding site. They found 2,3-DPG bound less readily to foetal haemoglobin in which histidine 143 (H21) on the non-alpha subunit is replaced by serine (Schroeder, Shelton, Shelton, Cormick and Jones, 1963) and this result suggested that the DPG-binding site was on the dyad axis of symmetry in the internal cavity between the two β-chains and involved histidine 143β. The α-amino group of valine 1 β (NA 1) is also present in this cavity and Bunn and Briehl (1970) demonstrated its involvement with 2,3-DPG binding, since 2,3-DPG had a considerably diminished effect on the oxygen affinity of foetal haemoglobin and glycosylated haemoglobin, where the N-terminal amino groups of the non-alpha chains are bound to an acetyl group and hexose respectively. The involvement of the α-amino groups was confirmed independently by Caldwell, Nagel and Jaffe (1971) and Tomita and Riggs (1971) who found the formation of carbamate by CO_2 at the α-amino groups of haemoglobin reduced the effect of 2,3-DPG on the oxygen dissociation curve.

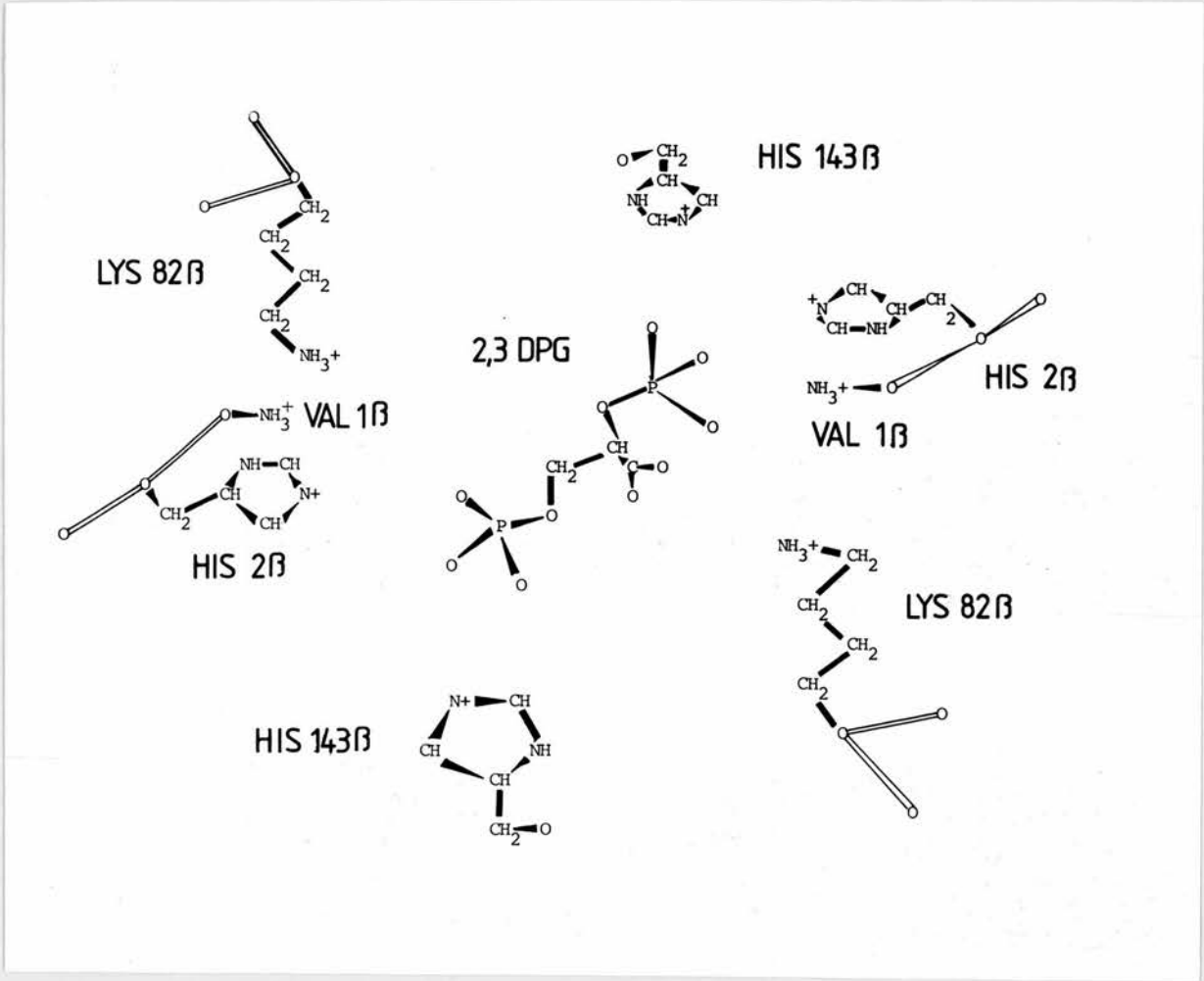


Figure 2

The binding position of the 2,3-DPG molecule in the human deoxy-haemoglobin structure. The negative charges on each phosphate group are shared between the residues valine 1 and histidines 2 and 143 in the β-subunits. (Arnone, 1972).

Perutz (1970), using x-ray crystallographic data, found that if a model of 2,3-DPG was placed on the dyad axis of symmetry of a model of reduced haemoglobin, the charged groups on the 2,3-DPG molecule were within hydrogen-bonding distance of the N-terminal valines, lysine 82 (EF6) and histidine 143 (H21) of the β-chains. Furthermore, on oxygenation, the distance between the α-amino groups increases, disrupting the hydrogen-bonds with 2,3-DPG, and the central cavity narrows, thus expelling 2,3-DPG and accounting for the lower affinity of 2,3-DPG for oxyhaemoglobin.

In 1972, Arnone succeeded in producing a difference electron density map between the human deoxyhaemoglobin-DPG complex and normal deoxyhaemoglobin which confirmed the binding of 1 mole 2,3-DPG to 1 mole haemoglobin at the entrance to the central cavity, which involves hydrogen-bonding to seven cationic groups of the β-chains: valines 1 (NA1), histidines 2 (NA2) and 143 (H21), and one of the lysines 82 (EF6) (Fig. 2).

Regulation of 2,3-DPG Concentrations

The pathway for the formation of 2,3-DPG was found by Rapoport and Luebering in 1950-52, and is now known as the Rapoport-Luebering shunt (Fig.3). 2,3-DPG is synthesised from 1,3 diphosphoglycerate by the action of diphosphoglycerate mutase (DPG-mutase), and is decomposed to 3-phosphoglycerate and inorganic phosphate by 2,3-diphosphoglycerate phosphatase (DPG-phosphatase). Both these reactions are irreversible and involve the same enzyme protein for catalysis. Thus, this cycle bypasses the phosphoglycerate kinase reaction in the glycolytic pathway and consequently no ATP is formed. Approximately 20% of the glycolytic flux is through this cycle, and the turnover rate of

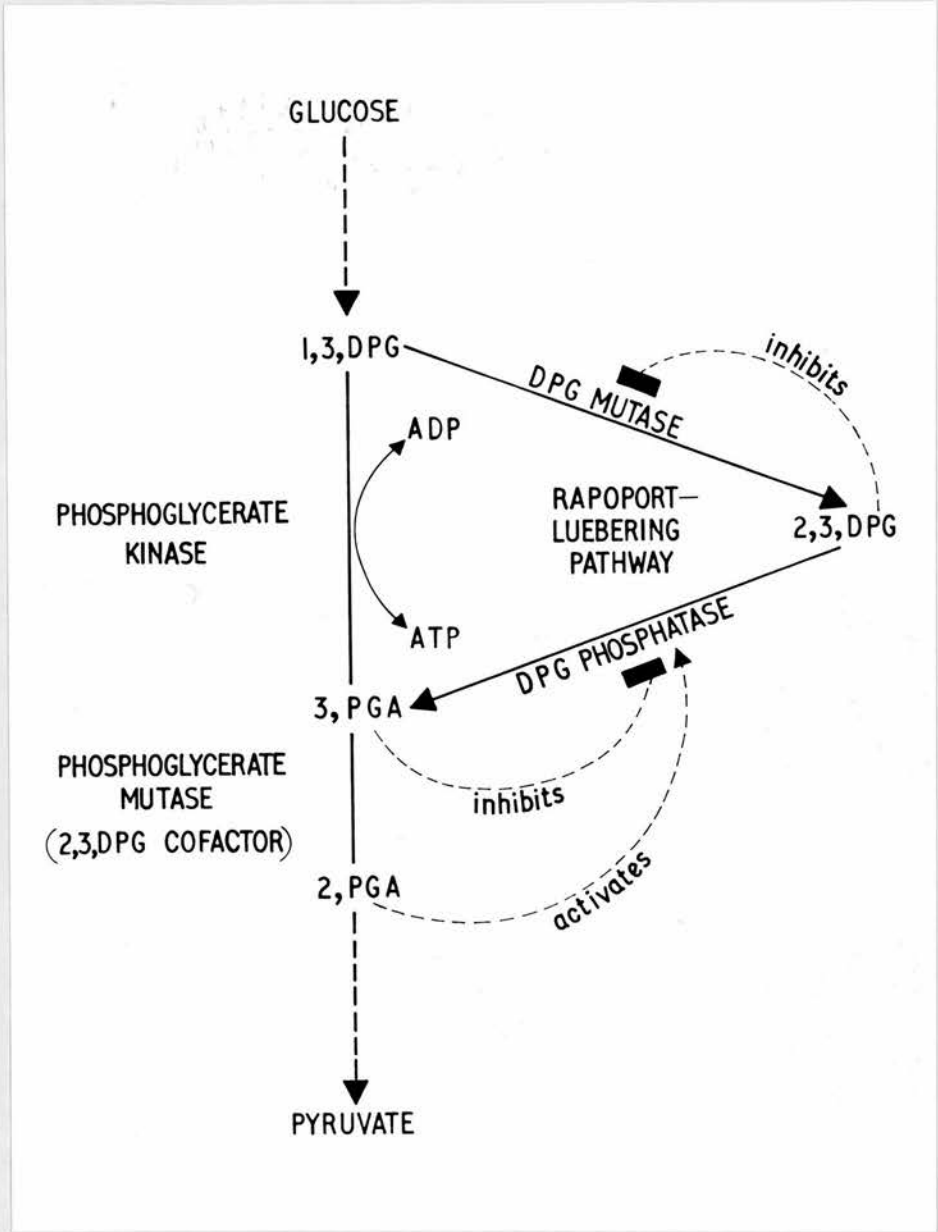


Figure 3

The Rapoport-Luebering pathway for the synthesis of 2,3-DPG, and its position in the main Embden-Meyerhof pathway.

2,3-DPG was calculated to be 0.3 - 0.75 μmoles per hour per millilitre of cells (Chiba and Sasaki, 1978).

Various factors control the concentration of 2,3-DPG under physiological conditions. In 1952, Rapoport and Luebering reported a negative feedback mechanism whereby 2,3-DPG inhibits the enzyme DPG-mutase when concentrations rise above the optimum level.

Hydrogen ion concentration has a considerable influence on 2,3-DPG concentrations. In alkalosis, the rate of glycolysis and the activity of DPG-mutase is increased and the activity of 2,3-DPG phosphatase inhibited, leading to an elevation of 2,3-DPG concentrations. In acidosis these changes are reversed with a subsequent reduction in 2,3-DPG levels. However, a change in pH would appear to exert opposing influences on 2,3-DPG levels, as a rise in pH, for example, would increase DPG-mutase activity and increase 2,3-DPG synthesis, but it would also lower the amount of 2,3-DPG bound to haemoglobin, increasing the concentration of free 2,3-DPG, which would, in turn, inhibit DPG-mutase activity, illustrating the complex regulatory role of pH.

The ADP/ATP ratio will decide whether 1,3-diphosphoglycerate enters the Rapoport-Lueberingshunt or proceeds through the glycolytic pathway. A high ratio facilitates the flux through the ATP synthesising step in the glycolytic pathway, whereby less 2,3-DPG will be formed.

Inorganic phosphate concentration can also regulate 2,3-DPG levels, as shown by Lichtman, Miller, Cohen and Waterhouse (1971), who found decreased levels of 2,3-DPG in hypophosphataemia, and elevated levels in hyperphosphataemia.

As the target for the regulation of the pathway must be the activity of free 2,3-DPG, the metabolism will tend to preserve a certain level in the free form. Thus, as reduced haemoglobin binds more 2,3-DPG than oxyhaemoglobin, it was anticipated by several authors (Benesch et al, 1968; Lenfant et al, 1968) that the level of 2,3-DPG depends upon the oxygenation of haemoglobin.

Lenfant et al (1968) found a marked rise in 2,3-DPG concentrations within 24 hours in subjects taken from sea level to 4530 m above sea level. This increased concentration was restored to normal on return to sea level and the authors related this effect to the reduced oxyhaemoglobin saturations and not to the alkalinity observed at altitude, for residents at high altitudes, brought to sea level, showed reduced levels of 2,3-DPG without any change in acid-base status. Studies by Oski, Gottlieb, Delivoria-Papadopoulos and Miller (1969) support this theory, although a direct relationship between oxygen saturation and 2,3-DPG levels was not observed. However, in 1970, they were able to show a correlation between 2,3-DPG levels and arterial oxygen tension, in patients with hypoxaemia due to congenital heart disease. However, normal 2,3-DPG levels were observed by Gallagher (1971) in asthmatic patients with low arterial oxygen tensions. Edwards and Canon (1972) similarly found normal 2,3-DPG levels in patients with chronic obstructive lung disease with associated acidosis. Fairweather, Walker and Flenley (1974), investigating 2,3-DPG levels in patients with chronic obstructive lung disease found the average 2,3-DPG concentration of the group was normal, although values both above and below the normal range were encountered, showing

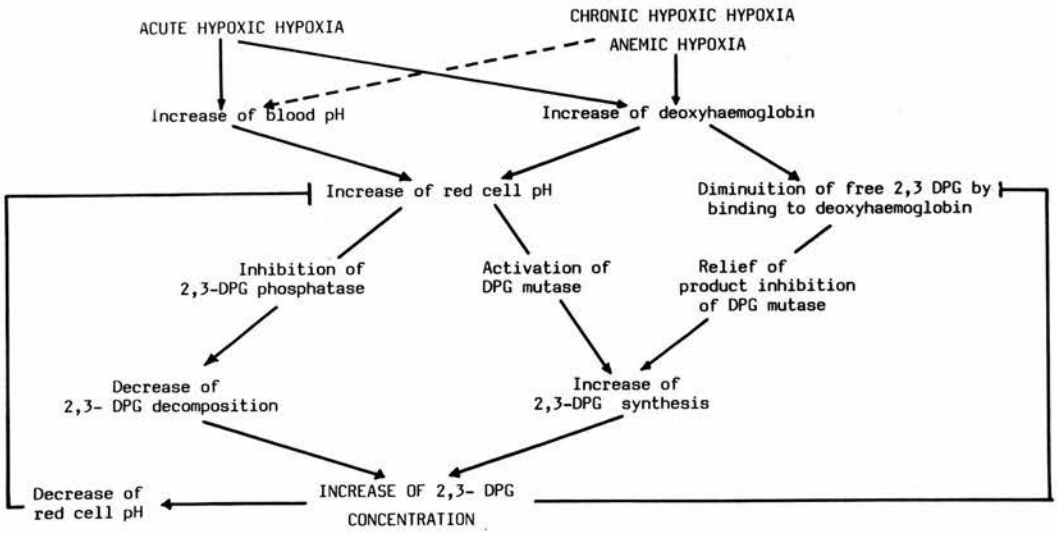


Figure 4

A schematic summary of mechanisms influencing 2,3-DPG concentration changes in the red blood cells during hypoxia. (Duhm and Gerlach, 1971).

the variability of response of 2,3-DPG to hypoxic stimulus (fig 4).

In 1971, Duhm and Gerlach investigated the effect of oxyhaemoglobin saturation on the synthesis and breakdown of 2,3-DPG in vitro, and found the rate of decomposition of 2,3-DPG was independent of oxygen saturation, but synthesis increased markedly in deoxygenated red cells. They suggested this may be due to the more alkaline intracellular pH on deoxygenation which would activate DPG-mutase and inhibit DPG phosphatase.

2,3-DPG levels are also influenced by exercise. Kunski and Sztobryn (1976) observed lower concentrations immediately following exercise in trained subjects, and elevated concentrations following exercise in untrained subjects. Ramsey and Pipoly (1979) also investigated 2,3-DPG levels after exercise, but in contrast to Kunski et al, found 2,3-DPG levels fell after exercise in untrained subjects. They attributed the fall to increased lactate concentrations causing acute acidosis with subsequent reduction in 2,3-DPG synthesis.

There is controversy, also, regarding the effect of age on 2,3-DPG. In 1974, Purcell and Brozovic reported a progressive decrease of 2,3-DPG concentration with advancing age, but Tweeddale, Leggett and Flenley (1976) found no such correlation in 62 subjects ranging in age from 18 - 89 years.

iii) Carbon Monoxide

Humans have always been exposed continuously to small quantities of carbon monoxide endogenously produced from the normal catabolism of haemoglobin, with a minor fraction contributed by the breakdown of non-haemoglobin haem. In healthy males at rest, the average rate of endogenous carbon monoxide production is approximately 0.4 ml per hour, resulting

in a carboxyhaemoglobin (COHb) saturation of 0.4 - 0.7%, which has been considered neither beneficial nor harmful (Stewart, 1976).

Most exogenous carbon monoxide is produced from the incomplete oxidation of carbonaceous material, the major source in the environment being the exhaust of motor vehicles and the remainder resulting from industry. Tobacco smokers are the most heavily exposed non-industrial segment of the population, and some may have COHb levels as high as 20%.

Modern studies on the interaction of carbon monoxide with haemoglobin can be said to date from the work of Claude Bernard in 1857, when it was shown that carbon monoxide displaces oxygen from haemoglobin. Haldane and Lorrain Smith (1897) investigated the combination of oxygen and carbon monoxide with ox haemoglobin, and concluded that when haemoglobin was saturated with oxygen and carbon monoxide, the relative proportions of oxy-and COHb are proportional to the relative partial pressures of the two gases, allowing for the fact that the affinity of carbon monoxide for haemoglobin is approximately 300 times greater than that of oxygen.

However, in 1897, the oxygen dissociation curve was thought to be a rectangular hyperbola, and the influence of carbon dioxide and salts on the curve was unknown. This prompted Douglas, Haldane and Haldane (1912) to reinvestigate the combination of oxygen and carbon monoxide with haemoglobin, in the light of subsequent discoveries regarding oxygen-haemoglobin interactions. They found the conclusions of Haldane and Lorrain Smith were correct and applicable even in the presence of various carbon dioxide concentrations. They constructed

equilibrium curves for carbon monoxide and haemoglobin combination and found them to have the same sigmoidal form as those for oxygen and haemoglobin combination, and they proposed that COHb dissociation curves could be made to coincide with the corresponding oxygen dissociation curves over their whole range, merely by altering the scale of carbon monoxide partial pressures. However, Joels and Pugh (1958) studied COHb dissociation curves using more accurate methods, and although they found excellent agreement between COHb and oxyhaemoglobin dissociation curves over the mid saturation range, at high saturations, the COHb curves lay slightly below the oxyhaemoglobin curves.

In their classic study, Douglas et al (1912) found that when COHb is present in blood, oxygen is given off from oxy-haemoglobin to the tissues in an abnormal way. This observation was investigated further by Stadie and Martin (1925) and Roughton and Darling (1944) who studied the effect of a wide range of COHb levels on the oxygen dissociation curve. As a result of this work, it has been generally accepted that partial saturation of haemoglobin with carbon monoxide not only decreases the amount of oxygen which haemoglobin can carry, but also shifts the oxygen dissociation curve of the remaining haemoglobin progressively to the left, indicating an increase in the affinity of haemoglobin for oxygen, and alters the slope of the curve, making it less S-shaped. Okada, Tyuma, Ueda and Sugimoto (1976) found that Hill's 'n' (a measure of the haem-haem interaction obtained from the slope of the Hill plot) gradually decreases as COHb levels are raised, falling from 2.6 at 0% COHb to 1.4 at 60% COHb.

These authors suggested that the effect of carbon monoxide in increasing the oxygen affinity of haemoglobin can be explained according to the intermediate compound hypothesis of Adair (1925). The hypothesis states that the combination of haemoglobin with oxygen or carbon monoxide takes place in four steps, and the association constant for each step increases as the ligand is bound, indicating a rise in haemoglobin oxygen affinity. Therefore, if the combination of haemoglobin with carbon monoxide takes place in a similar manner to that with oxygen, and the partially saturated carboxyhaemoglobin is assumed to be equivalent to the partially oxygenated haemoglobin, then increased fractions of carboxyhaemoglobin should lead to a higher oxygen affinity of the remaining haemoglobin.

For these reasons, the hypoxic symptoms produced by carbon monoxide intoxication are more severe than those produced by anaemia, despite the same blood oxygen content (Fig. 5).

In their study, Roughton and Darling (1944) developed a theoretical procedure for calculating the position of the oxygen dissociation curve in the presence of COHb, and this has shown agreement with experimental results (Okada et al, 1976).

In addition to its effect on the oxygen dissociation curve, carbon monoxide affects other factors associated with oxygen transport. In 1956, Eisen and Hammond, investigating the effects of elevated COHb in smokers, found that haematocrit, red cell count and haemoglobin concentrations were higher during periods of cigarette smoking than during abstinence periods, but the time course of these responses was not clearly elucidated. Wilks, Tomashefski and Clark (1959) exposed dogs to carbon monoxide for 6 - 8 hours daily for 36 weeks and found their tolerance

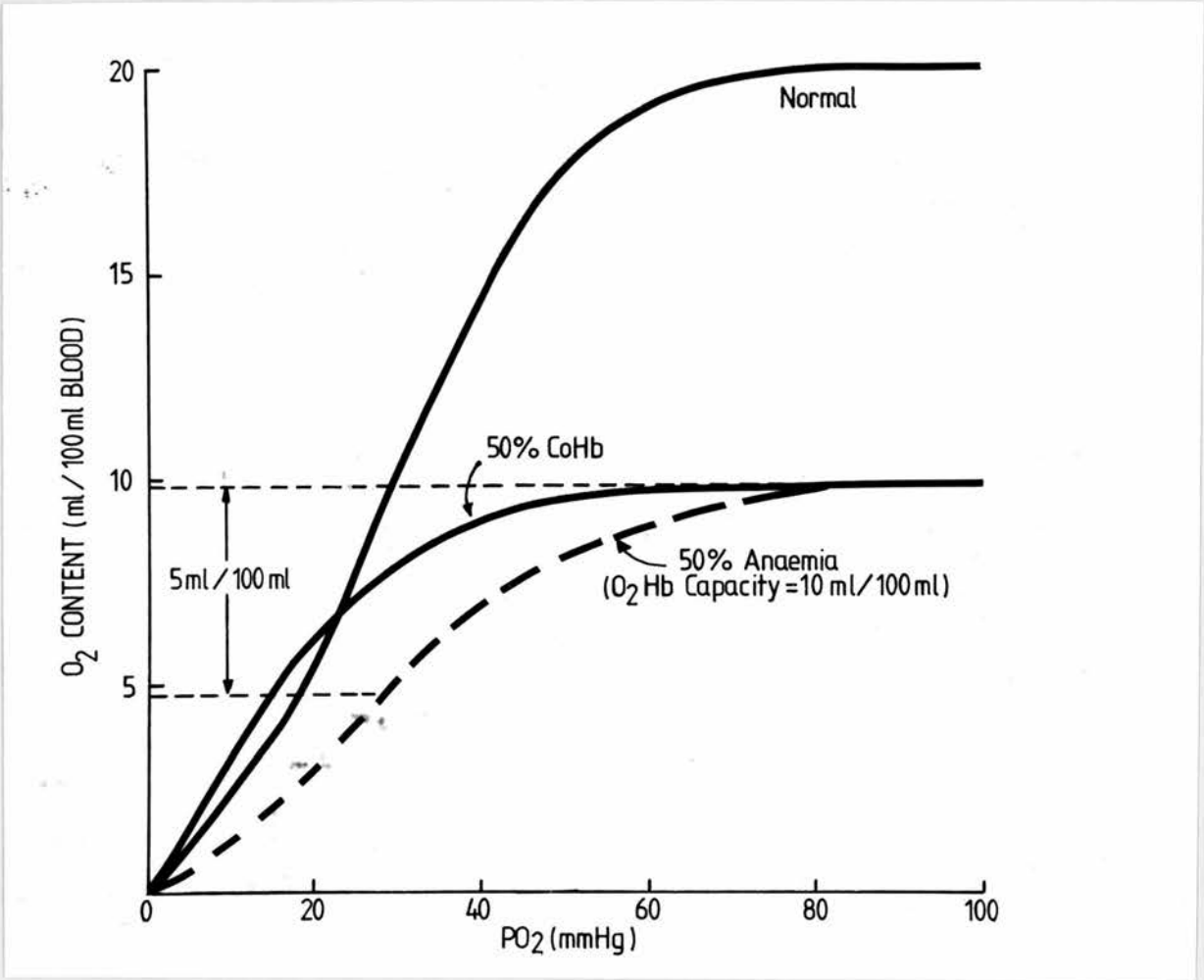


Figure 5

Oxyhaemoglobin dissociation curves of normal human blood, blood containing 50% COHb, and blood containing 50% of the normal amount of haemoglobin, showing that for any given arterio-venous O_2 content difference, the subject with 50% COHb will have a lower venous PO_2 than the subject with 50% anaemia. (Bartlett, 1968).

to carbon monoxide was increased. They attributed this to an increase in the concentration of haemoglobin, which would produce a greater oxygen capacity at any COHb concentration. Later investigations on humans, showed an immediate post-carbon monoxide exposure rise in haemoglobin and haematocrit, which subsided within 48 hours and could be attributed to a fall in plasma volume, together with a delayed, more prolonged rise in haemoglobin and haematocrit related to erythropoietic activity (Ramsey, 1969).

Cigarette smokers, who are chronically exposed to low levels of carbon monoxide, have been investigated by several groups. Sagone, Lawrence and Balcerzak (1973) found significantly higher haematocrit, haemoglobin, red cell counts and red cell mass in a group of cigarette smokers, despite normal arterial oxygen tensions. The erythropoietic response was attributed to lower arterial oxygen saturations, expressed as a percentage of total haemoglobin (Sagone and Balcerzak, 1975) and this was confirmed by Smith and Landaw (1978). It is believed that hypoxia at some site within the kidney releases erythrogenin, an enzyme able to act on one of the plasma proteins to produce erythropoietin, which, in turn, stimulates the bone marrow to increase erythropoiesis (Gordon and Zanjani, 1970).

In view of the leftward shift of the oxygen dissociation curve induced by carbon monoxide, a subsequent effect of carbon monoxide on 2,3-DPG concentrations has been sought. Dinman, Eaton and Brewer (1970) exposed normal human volunteers and also rats to carbon monoxide at two levels of exposure. The treated rats showed marked elevation of 2,3-DPG with the low carbon monoxide exposure, but no change with the high exposure.

Similarly, 2,3-DPG levels increased in human subjects given the low dose of carbon monoxide, but there was no change in subjects given the larger dose. The authors were unable to account for these results using existing theories of 2,3-DPG regulation and suggested that hypoxia caused by carbon monoxide triggers some other mechanism which elevates 2,3-DPG. However, in the same year, Astrup (1970) investigated the effect of similar COHb levels over a period of 24 hours, and observed a fall in 2,3-DPG concentrations, which he attributed to a change in red cell pH resulting from the decrease in reduced haemoglobin when COHb levels rise. Other investigators have found 2,3-DPG is totally unaffected by elevation of COHb in both acute and chronic exposure studies (Sagone et al, 1973; Ramsey and Casper, 1976; Thomas and Penney, 1977; Smith et al, 1978).

Okada et al (1976) reversed the emphasis and investigated the effect of 2,3-DPG on the shift of the oxygen dissociation curve produced by COHb and found the shift in the curve was constant in the presence and absence of 2,3-DPG. They also found that COHb had no effect on the fixed acid Bohr effect, a result which was contested by Hlastala, McKenna, Franada and Detter (1976) who found the fixed acid and CO_2 Bohr effects increased as COHb levels rose, but were unable to explain the difference of results.

As carbon monoxide is so detrimental to oxygen transport, it is to be expected that exercise capacity will be impaired by high COHb levels. In 1910, Nicolai and Staehelin determined upon themselves the effect of smoking, and found an increase in heart rate and blood pressure after a measured amount of work. Juurup and Muido (1946) measured work capacity after smoking,

and again found increased heart rates, but COHb levels were not monitored during these studies.

From more recent studies, a number of investigators have shown a linear decline in aerobic power, or maximal oxygen uptake ($\dot{V}O_2$ max), as COHb levels rise (Ekblom and Huot, 1972; Vogel and Gleser, 1972; Pirnay, Dujardin, Deroanne and Petit, 1971) so that the subject with higher COHb levels is effectively working at a higher relative work load, and the critical level of COHb above which maximum aerobic power would be impaired was found to be as low as 4.3% COHb (Horvath, Raven, Dahms and Gray, 1975).

Oxygen Transport in Chronic Bronchitis

By the 19th century, the clinical diagnosis of emphysema and chronic bronchitis with chronic ventilatory failure was well known. Chronic ventilatory failure (Type II respiratory failure) is defined as being present when the arterial CO_2 tension is chronically elevated above the normal range, and the arterial O_2 tension is below the normal range. In order to maintain an adequate supply of oxygen to the tissues, numerous adaptive mechanisms are employed, and the mechanisms affecting the relationship between haemoglobin and oxygen are discussed.

There is much uncertainty about changes in the oxygen affinity of haemoglobin which occur in chronic obstructive airways disease. Edwards, Novy, Walters and Metcalfe (1968) and Lyons and Tabak (1972) found increased P50 values (corrected to pH 7.4 ($P50_{(7.4)}$)) in all patients with chronic airways obstruction. However, Lenfent, Ways, Aucutt and Cruz (1969) found the situation less clear-cut, and that the oxygen affinity

depended on the haematocrit, being increased in patients with normal haematocrit values, but reduced in patients with a haematocrit greater than 50%; the P50 (7.4) increasing with the degree of polycythaemia. In a more recent study, Denis, Feret, Nouvet, Pasquis, Stain, Weisang, Morere and Lefrancois (1977) found increased P50 (7.4) values only in patients with a comparative anaemia, a result contrary to that of Lenfant et al (1969).

On the other hand, a number of studies indicate no consistent alteration of oxygen affinity from the normal value in patients with chronic obstructive airways disease, despite severe hypoxaemia (Guy, Kazura, Bromberg and Balcerzak, 1971; Astrup, 1969; Edwards and Canon, 1972; Fairweather, Walker and Flenley, 1974). However, in these studies no attempt was made to compare the P50 values of the patients with those of similarly aged healthy subjects, and as P50 (7.4) was found to increase with age (Tweeddale, Leggett and Flenley, 1976), Tweed dale, Leggett and Flenley (1977) compared oxygen affinity in patients, with that in age-matched normal subjects. They found no difference in P50 (7.4) between the two groups, although the value was higher than that given by the standard oxygen dissociation curve of Severinghaus (1966).

The Bohr effect in patients with airways obstruction was found to lie within the normal range (Edwards et al, 1968; Lenfant et al, 1969), but Edwards et al (1968) found haem-haem interaction was increased in these patients, although Lenfant et al (1969) found it to be normal.

Concentrations of 2,3-DPG in these patients have also been reported as altered, or unchanged. Oski, Gottlieb,

Delivoria-Papadopoulos and Miller (1969) found 2,3-DPG concentrations were significantly higher in patients with chronic bronchitis, but many studies have shown normal 2,3-DPG concentrations, despite hypoxaemia in these patients (Guy et al, 1971; Edwards and Canon, 1972; Fairweather et al, 1974; Tweeddale et al, 1977 and Denis et al, 1977). Respiratory acidosis may account for the lack of elevation of 2,3-DPG concentrations in some patients with chronic obstructive airways disease.

It must be remembered that in patients with respiratory acidosis, the P50 in vivo will be higher than the corresponding $P50_{7.4}$ due to the Bohr effect. However, this benefit of a lower oxygen affinity may be curtailed if the low pH acts to reduce 2,3-DPG levels, but such a fall in 2,3-DPG below normal levels was not observed in any of these studies, and the rightward shift of the oxygen dissociation curve in vivo, due to the Bohr effect was, therefore, not counterbalanced. Edwards et al (1972) estimatedfrom their results that the mean in vivo P50 value would be 30 mm Hg in patients with chronic obstructive lung disease and acidosis.

Although this rightward shift in the oxygen dissociation curve was held to be an important adaptation in hypoxia, Flenley, Fairweather, Cooke and Kirby (1975) showed that large variations in P50 had comparatively little effect on oxygen transport, assessed by venous oxygen tensions, when hypoxic patients were breathing air. They explained this paradox as being due to the shape of the dissociation curve. When the arterial O_2 tension lies below the shoulder of the curve, two patients with widely differing P50 values have similar venous

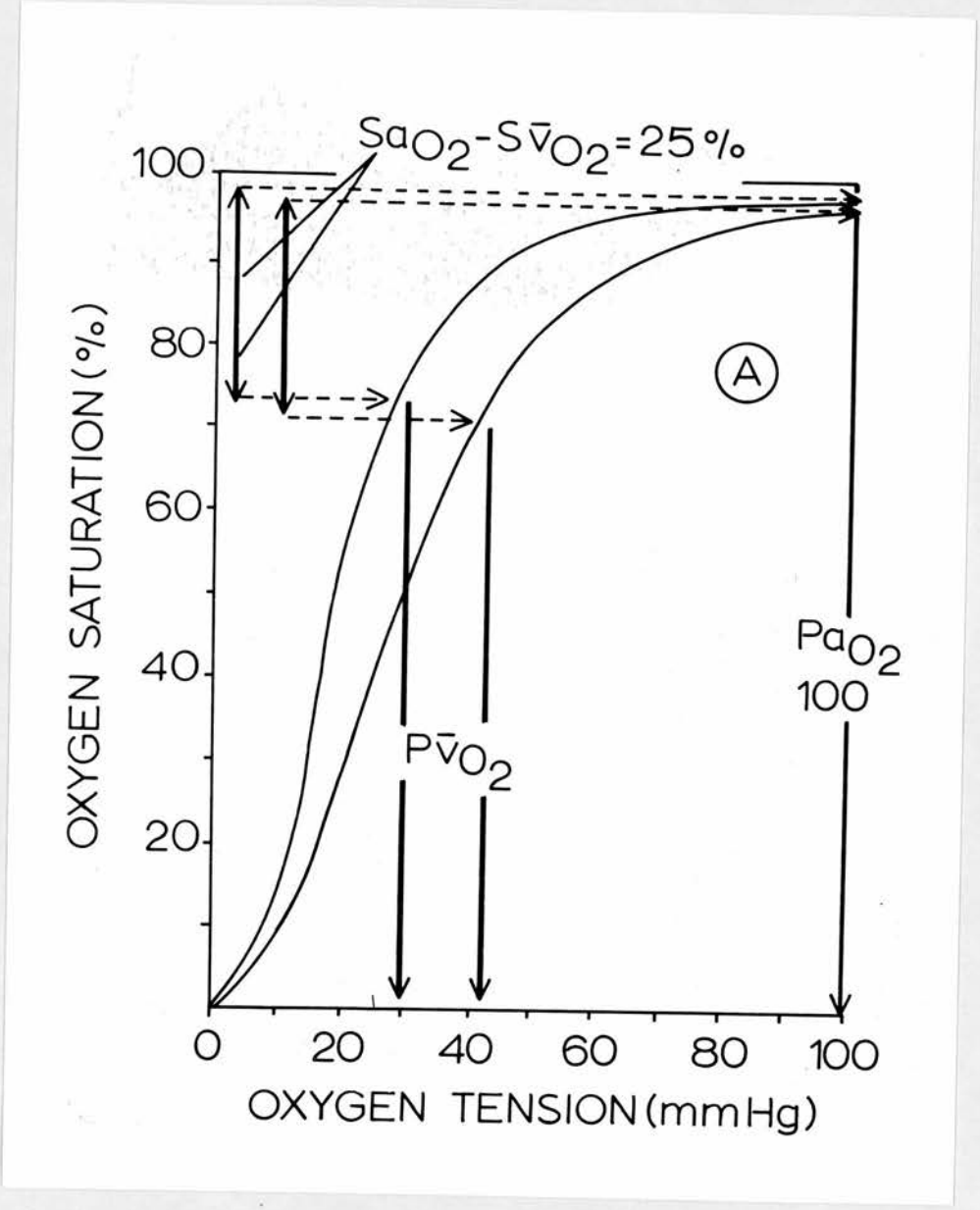


Figure 6a

Oxyhaemoglobin dissociation curves with $P50_{(7.4)}$ values of 20 mm Hg (left curve) and 30 mm Hg (right curve). With arterial $P0_2$ = 100 mm Hg and an arterio-venous 0_2 saturation difference of 25%. there is considerable difference in terms of venous $P0_2$ between the two curves. (Flenley, Fairweather, Cooke and Kirby, 1975).

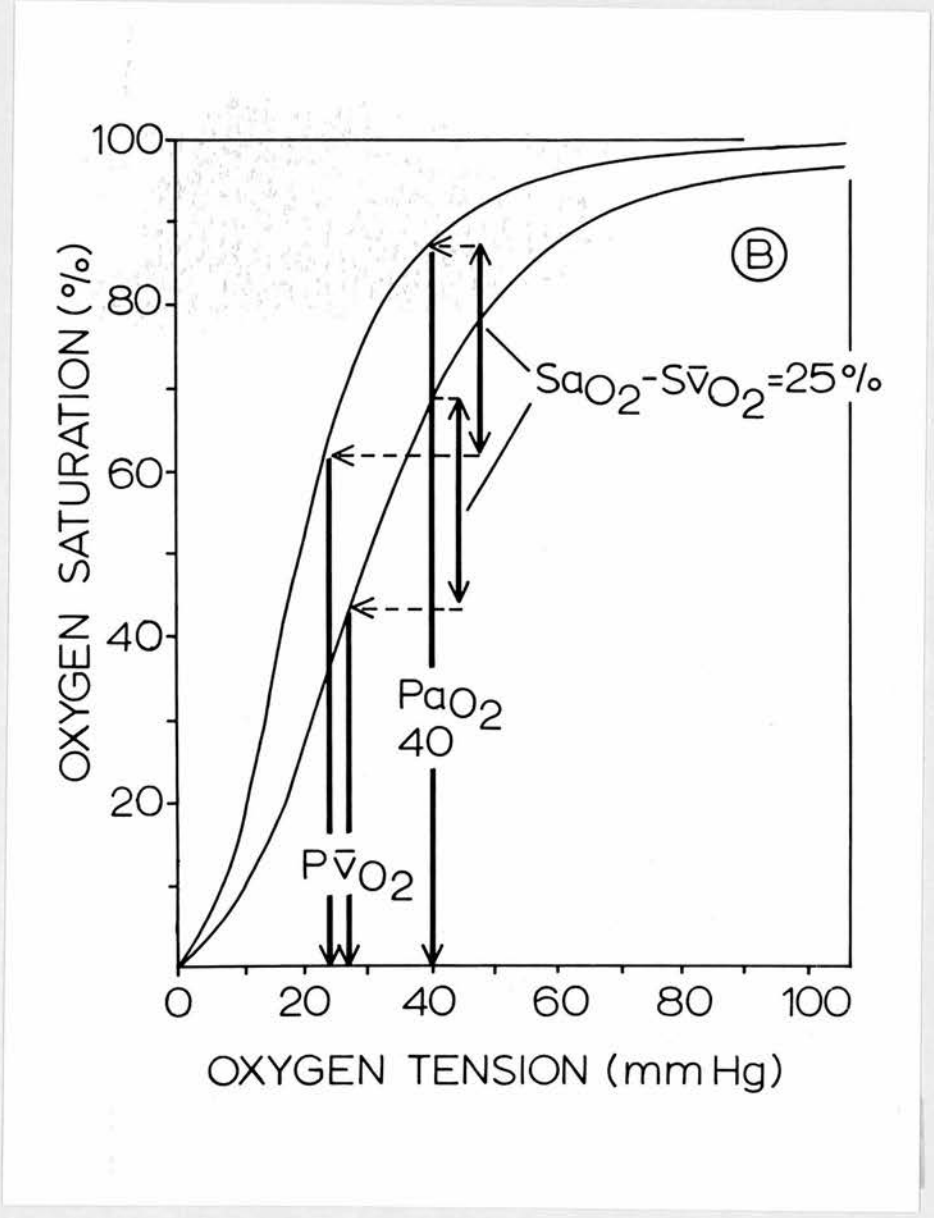


Figure 6b

Oxyhaemoglobin dissociation curves with $P50_{(7.4)}$ values of 20 mm Hg (left curve) and 30 mm Hg (right curve). With arterial PO_2 = 40 mm Hg and an arterio-venous O_2 saturation difference of 25%, the venous points on the curves are close in terms of PO_2. (Flenley, Fairweather, Cooke and Kirby, 1975).

0_2 tensions for a given arterial-venous 0_2 content difference, ($\Delta(A\text{-}V)0_2$). However, when the arterial 0_2 tension lies over the shoulder of the curve, the venous 0_2 tensions differ markedly, the patient with the higher P50 having the advantageous value. Thus, in patients with low arterial 0_2 tensions, the shift in the curve produced by elevated 2,3-DPG levels will afford little advantage to tissue oxygenation (Figs.6a and 6b).

A further adaptive mechanism to maintain tissue oxygenation is an increase in red cell production. It was originally believed that hypoxaemia is generally not accompanied by a rise in haemoglobin concentration (Baldwin, Cournand and Richards, 1949; Wilson, Borden and Ebert, 1951). However, Vanier, Dulfano, Wu and Desforges (1963) demonstrated that it is, in fact, accompanied by an increase in red cell mass which, because of changes in plasma volume, is not always reflected in a rise in haemoglobin or haematocrit. An inverse relationship was subsequently demonstrated between arterial oxygen saturation and red cell mass in patients with chronic airways obstruction (Weil, Jamieson, Brown and Grover, 1968; Chan, 1969; Harrison, 1973), but Guy et al (1971) found that only half of the patients that they studied had an elevated red cell mass, and suggested that the response is not stereotyped and may be modified by the underlying disease state.

Oxygen Therapy in Chronic Bronchitis

It has been recognised for many years that administration of oxygen to selected patients with chronic airways obstruction was of clinical benefit. Oxygen was first given therapeutically to patients with tuberculosis by Chaussier in 1780, and although

the clinical diagnosis of chronic bronchitis and emphysema was well known in the 19th century, the serious therapeutic use of oxygen for the treatment of the disease did not come until the early part of this century. Haldane (1919) suggested that long term oxygen therapy may benefit patients with chronic obstructive lung disease, but this was not investigated until 1926, when Campbell and Poulton studied patients residing for 1 - 3 weeks in a chamber containing 40% oxygen. Although the condition of the patients improved, it was not long-lasting, and no relief of the associated secondary polycythaemia was observed, as longer-term continuous oxygen therapy was difficult, due to the cumbersome equipment necessary.

In 1956, Cotes and Gilson described portable oxygen equipment which could be used in the home. They found from their study that administration of 30% oxygen was almost as beneficial to the patients as 50% or 100% oxygen, and would minimise the toxic effects of oxygen. Long term oxygen therapy for ambulatory patients became possible in 1967 following the introduction of lightweight apparatus for carrying liquid oxygen (Linde walker), and the value of such continuous oxygen therapy in patients with severe hypoxaemia has been demonstrated by many research groups. A reduction in haemoglobin concentration, haematocrit and red cell volume was observed following oxygen therapy for at least 12 hours per day over periods of time ranging upwards from six weeks (Chamberlain and Millard, 1963; Petty and Finigan, 1968; Leggett, Cooke, Clancy, Leitch, Kirby and Flenley, 1976; Block, Castle and Keitt,1974). Mean pulmonary artery pressure and pulmonary vascular resistance was reduced in some patients (Horsfield, Segel and Bishop, 1968;

Levine, Bigelow, Hamstra, Beckwitt, Mitchell, Nett, Stephen and Petty, 1967; Leggett et al, 1976), and Block et al (1974) observed a fall in 2,3-DPG concentration following oxygen therapy for four weeks.

However, correction of hypoxaemia by oxygen administration to patients with chronic obstructive bronchitis may lead to carbon dioxide retention (Donald, 1949; Westlake, Simpson and Kaye, 1955), a phenomenon attributed to the removal of a ventilatory stimulus from hypoxia, whereas a rise in CO_2 tension is a relatively inefficient stimulus to ventilation in these patients.

The Transport of CO_2 by the Blood

The transport of CO_2 by the blood has been of interest to physiologists for over a century, and by the beginning of this century it was known that most of the CO_2 in the blood was in loose chemical combination, the plasma holding about two-thirds, and the red cells about one-third of the total. Much of this loose chemical combination of CO_2 was known to take place via reactions shown:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad (1)$$

$$H_2CO_3 + NaA \rightleftharpoons HA + NaHCO_3 \qquad (2)$$

where A represents phosphates or other such substances.

In 1914, Christiansen, Douglas and Haldane established the shape of the CO_2 dissociation curve, and having constructed curves for fully oxygenated and reduced blood, found that oxygenated blood carried less CO_2 than reduced blood at any given PCO_2 value. They suggested this was because oxyhaemoglobin was a stronger acid than reduced haemoglobin which caused equations (1) and (2) above to move to the left. They were unable to prove this at the time, however, as pH could not be measured accurately.

On the basis of their results, they proposed that this O_2-linked CO_2 expulsion would contribute 30 - 40% of the CO_2 expelled in the lungs, and, as such, was of great physiological importance. It was later demonstrated that the shape of the CO_2 dissociation curve and position can vary between individuals (Liljestrand and Lindhard, 1920; Meakins and Davies, 1922), and is dependent upon the haemoglobin concentration (Brocklehurst, Haggard and Henderson, 1927).

The idea of a direct combination of CO_2 and haemoglobin was first suggested by Bohr, but no evidence for this compound was produced until, in 1928, Henriques found that when haemoglobin solutions were shaken in vacuo, CO_2 was liberated very rapidly to begin with, and then at a slower rate similar to that found when plasma was shaken in vacuo. He proposed the first rapid phase was due to the dissociation of a carbamate-type compound of CO_2 and haemoglobin, and the slow phase to the uncatalysed reaction:

$$H^+ + HCO_3^- \rightleftharpoons H_2O + CO_2 \qquad (3)$$

However, by 1932 an enzyme for the above reaction had been isolated by Meldrum and Roughton, and given the name carbonic anhydrase.

Further support was given to Henriques suggestion of a reversible carbamate compound of CO_2 and haemoglobin by Margaria and Green in 1933. They showed that the first ionisation constant of carbonic acid changed when haemoglobin was added to the solution and suggested this was due to a combination of some CO_2 with haemoglobin. However, the matter was finally settled by Ferguson and Roughton in 1934. They developed a method for the quantitative estimation of CO_2 bound to ox haemoglobin, and produced evidence to show that CO_2 binds

as carbamate to $-NH_2$ groups in the haemoglobin molecule. They then investigated the effect of pH and oxygen saturation on the formation of carbamates. A rise in pH from 7.8 to 8.6 increased the carbamino-bound CO_2 almost sixfold, a result which was expected, as Faurholt, in 1925, had carried out experiments on carbamino compounds of amino acids, and found the following reactions were involved:

$$PNH_3^+ \rightleftharpoons PNH_2 + H^+ \qquad (4)$$

$$PNH_2 + CO_2 \rightleftharpoons PNHCOOH \qquad (5)$$

$$PNHCOOH \rightleftharpoons PNHCOO^- + H^+ \qquad (6)$$

Hence a rise in pH would drive equations (4) and (6) to the right, increasing carbamate formation.

Oxygenation, under otherwise static conditions, caused a reduction in carbamino-bound CO_2, which they attributed to steric hindrance to the combination of CO_2 caused by the attachment of the neighbouring oxygen molecule. This oxygen-linked carbamate formation, together with the increase in pH which occurs in deoxygenation, accounts for the higher CO_2 content of deoxyhaemoglobin, first demonstrated by Christiansen, Douglas and Haldane in 1914 and which has become known as the Christiansen, Douglas, Haldane (C.D.H.) effect.

In a subsequent paper, Ferguson (1936) applied the same methods to human haemoglobin and calculated that in man carbamino-CO_2 is responsible for about 30% of the total changes in CO_2 in the whole blood during the respiratory cycle. In 1937 Stadie and O'Brien confirmed and extended the work of Roughton and Ferguson. Using horse and ox haemoglobin solutions, they verified Roughton and Ferguson's calculations as to the role of carbamates in the circulation and brought forth new evidence

suggesting that the increased carbamate formation in deoxyhaemoglobin is dependent on the same $-NH_2$ groups whose power of combination with H^+ ions is greatly altered by oxygenation and thus responsible for oxyhaemoglobin being a stronger acid.

However, in 1948, Wyman put the whole matter of oxygen-linked carbamate formation in some doubt by pointing out that if the above proposals were correct, the $-NH_2$ groups in deoxyhaemoglobin would be in the charged form, $-NH_3^+$, and therefore unable to combine with CO_2. Wyman's proposal was that CO_2 combined with the $\Sigma-NH_2$ groups which are not influenced directly by oxygenation, but whose power of carbamate formation is proportional to pH. He suggested that the greater carbamate formation of deoxyhaemoglobin is due purely to the rise in pH accompanying reduction, which he calculated would be about 0.1 pH units. Using some of Ferguson's data, Wyman showed that this pH change could account for the difference in carbamate formation between oxy and deoxyhaemoglobin.

However, Roughton, in 1949, pointed out that if all the data was used, it would not fit Wyman's hypothesis well.

The dispute rested until 1962, when Rossi and Roughton measured the pH change accompanying oxygenation and found it to be only half of Wyman's estimate, and thus could not account solely for the difference in carbamino-bound CO_2 observed by Ferguson in deoxy- and oxyhaemoglobin. Rossi-Bernardi and Roughton confirmed this result in 1967 and also demonstrated that reduced haemoglobin solutions contained more CO_2 than oxy-haemoglobin solutions at the same pH and PCO_2 levels, thus proving beyond doubt that in the physiological range, carbamates are oxylabile. This differenece in CO_2 content between oxy and

reduced haemoglobin was shown to increase as pH rose, an observation later confirmed in whole blood by Mithoefer, Thibeault and Bossman (1969). On the basis of these results, they were able to reconfirm the role of carbamate in the transport of CO_2 and calculated that 26.7% of the CO_2 carried by the circulating blood is carried as carbamate.

In 1969, Bauer reported that 2,3-DPG reduces the amount of CO_2 bound as carbamate in haemoglobin solutions, an observation subsequently confirmed by Pace, Rossi-Bernardi and Roughton (1970), who found the CO_2 content of reduced haemoglobin with 2,3-DPG present, was half that in 2,3-DPG depleted solutions. With this knowledge, Rossi-Bernardi and Roughton (1970) reassessed the role of carbamate in the transport of CO_2 by the blood, and calculated that carbamate in the erythrocytes may only play about half the role previously computed in physiological CO_2 transport. Bauer (1970) extended his previous studies and investigated directly the effect of 2,3-DPG on carbamate formation. He confirmed the above results and suggested that in the presence of 2,3-DPG there is a reduction in the binding sites for CO_2 rather than a direct competition, as it appears that 2,3-DPG binds to the amino groups of the β chains, only when they are in the charged $-NH_3^+$ form, where no carbamate could be formed.

In 1972, Bauer and Schröder estimated carbamino-bound CO_2 in adult and foetal red blood cells, and although the amount of carbamate present in oxygenated adult and foetal cells was the same, they found reduced foetal haemoglobin formed more carbamate compounds than reduced adult haemoglobin. This observation was consistent with Bauer's hypothesis, as foetal

haemoglobin has a lower affinity for 2,3-DPG than adult haemoglobin (De Verdier and Garby, 1969), and therefore antagonism to carbamate formation is low. The contribution of carbamate to physiological CO_2 transport was calculated and found to account for only 10.5% of the total CO_2 transported, a figure similar to that proposed by Rossi-Bernardi and Roughton (1970) having taken the influence of 2,3-DPG into account.

In 1973, Klocke also showed the antagonistic effect of 2,3-DPG on carbamate formation, and investigated the total CO_2 content change on oxygenation at various pH values, and divided this change into that due to the change in carbamate and that due to the change in bicarbonate. He found the total CO_2 change on oxygenation was maximum at pH 7.40 and remained constant up to pH 7.6, but the relative contributions of carbamate and bicarbonate varied markedly. For a PCO_2 of 42.5 mm Hg and a normal 2,3-DPG concentration, the change in carbamate on oxygenation equalled the change in bicarbonate, at a pH of 7.5. Above this, carbamate contributes most to the total CO_2 change, and below this pH value, bicarbonate contributes the greater part, hence at the normal pH of 7.4, 40% of the total CO_2 change is due to the change in carbamate and 60% to the change in bicarbonate (fig.7).

Although initially there was much controversy over which $-NH_2$ groups were involved in carbamate formation, in 1967 Rossi-Bernardi and Roughton produced evidence which supported the idea that it is terminal α amino groups, rather than Σ-amino groups that are responsible for carbamate formation. The identification of the groups responsible was undertaken subsequently by Kilmartin and Rossi-Bernardi (1969, 1971) using horse haemoglobin which had been modified at the α amino groups with cyanate, to give three derivatives:

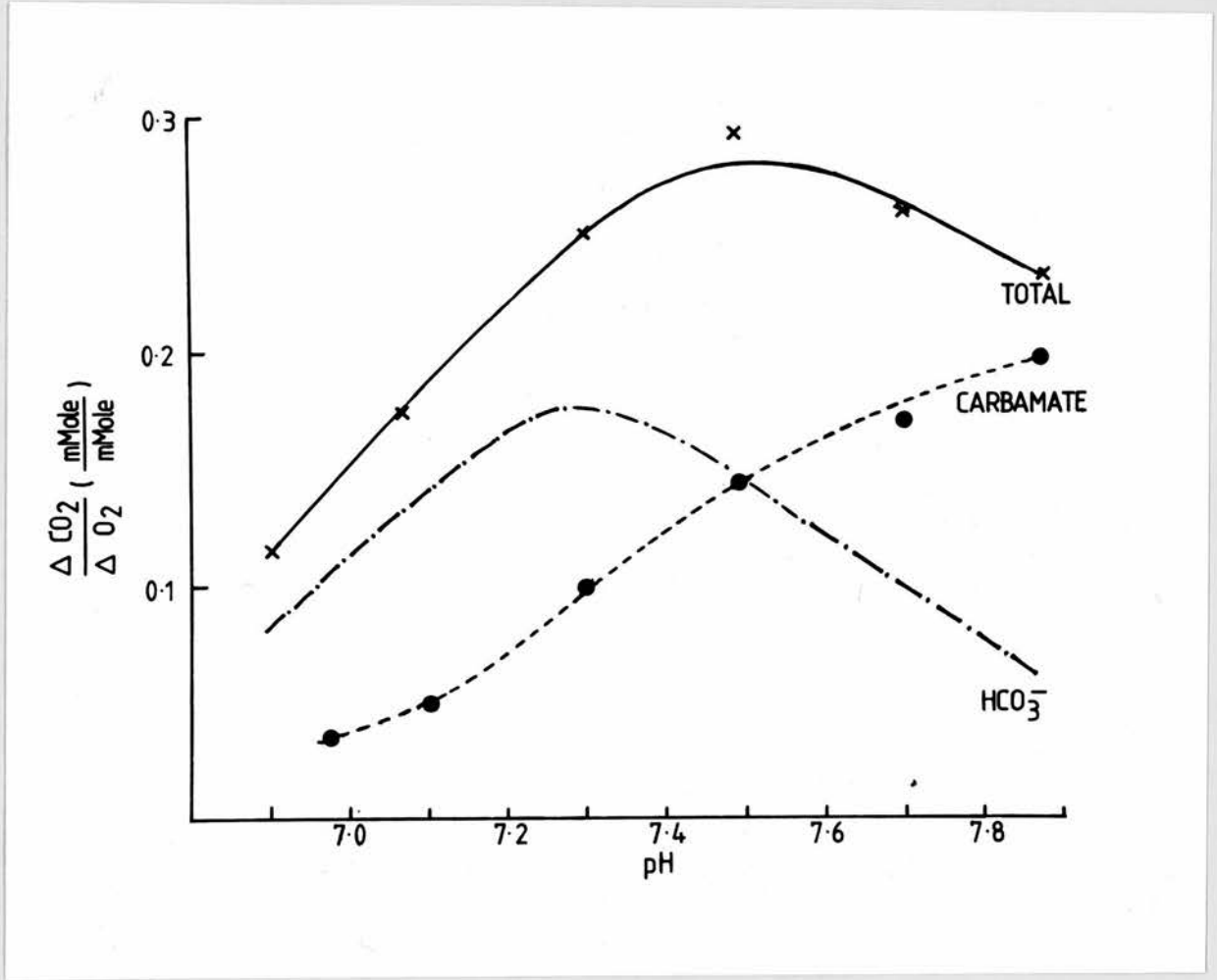


Figure 7

Variation in oxylabile carbamate, bicarbonate and total CO_2 content as a function of pH at a mean PCO_2 of 42.5 mm Hg. (Klocke, 1973).

$\alpha_2^c\beta_2^c$, $\alpha_2^c\beta_2$ and $\alpha_2\beta_2^c$, where superscript c denotes a specifically reacted α amino group. They determined carbamino-CO_2 indirectly by observing the change in oxygen affinity at constant pH, which is believed to be due to carbamate formation. In unmodified haemoglobin a large change in oxygen affinity was observed in the presence of CO_2, but in $\alpha_2^c\beta_2^c$, CO_2 had no effect on oxygen affinity, whereas $\alpha_2^c\beta_2$ and $\alpha_2\beta_2^c$ showed intermediate effects, confirming that all four N-terminal α amino groups are responsible for carbamate formation. This work was confirmed and extended by Kilmartin, Fogg, Luzzana and Rossi-Bernardi in 1973 using human haemoglobin modified in the same way. They found that CO_2 had a greater effect on the oxygen affinity of $\alpha_2^c\beta_2$ than on that of $\alpha_2\beta_2^c$, indicating there is a large change in the CO_2 binding constants of the β subunit α amino group between oxy and reduced forms of haemoglobin. In addition they found the change in oxygen affinity of $\alpha_2\beta_2^c$ caused by CO_2 was not diminished by the presence of 2,3-DPG, but the change in oxygen affinity of $\alpha_2^c\beta_2$ by CO_2 was markedly reduced after addition of 2,3-DPG, showing that 2,3-DPG interferes with the O_2-linked binding of carbon dioxide only at the α amino groups of the β-subunits.

In 1974, Arnone attempted to locate the binding of CO_2 to crystalline human deoxyhaemoglobin directly using x-ray studies. His work confirmed that CO_2 reacts with the N-terminal amino groups of the β chain, but was unable to show any combination with the α chain amino groups, which may have been due to the conditions used in the study. However, in 1975, Perrella, Kilmartin, Fogg and Rossi-Bernardi, and other workers (Bauer, Bauman, Engels and Pacyna, and Perrella, Bresciani and Rossi-Bernardi) showed that the β subunit N-terminal amino groups

have a threefold higher affinity for CO_2 than the ∝ chain N-terminal amino groups when DPG is absent, which may be explained with the help of results obtained by Matthew, Morrow, Wittebort and Gurd in 1977. They evaluated pKz, the negative log. of the ionisation constant for the reaction:

$$HbNH_3^+ \rightleftharpoons HbNH_2 + H^+ \qquad (7)$$

and also pKc, the negative log. of the equilibrium constant of the reaction:

$$HbNH_2 + CO_2 \rightleftharpoons HbNHCOO^- + H^+ \qquad (8)$$

for the N-terminal ∝ amino groups of the ∝ and β subunits. These values revealed that the N-terminal ∝ amino groups of the ∝ subunits are primarily protonated in deoxyhaemoglobin, and therefore much less free to form carbamino derivatives than the N-terminal ∝ amino groups of the β subunits which are primarily unprotonated at physiological pH. They also found that as haemoglobin is oxygenated, pKc for the β-chain N-terminal ∝ amino groups is raised, which causes equation (8) to move to the left, and thereby explains why the N-terminal ∝ amino groups of the β-chains form and discharge more carbamate than those groups of ∝-chain. In the same year, Bauer and Kurtz (1977) showed that a tetrameric molecular structure is a prerequisite for the binding of oxygen-linked carbamate, as no carbamate formation was detectable in monomers despite the presence of free terminal ∝ amino groups.

Summary

Haemoglobin is a tetrameric protein molecule comprising two α and two β subunits. Each subunit contains a haem group, at the centre of which is an iron atom which can combine reversibly with oxygen or other ligands. The tertiary structures of the α and β subunits are similar, consisting of seven helical regions in the α subunit, and eight in the β subunit. The quaternary structure of haemoglobin varies as ligands bind and dissociate, this change playing an important part in the functional role of haemoglobin.

Based on comparisons of electron density maps at 2.8 Å resolution, and of atomic models of deoxy and liganded haemoglobin, structural explanations of haemoglobin function have been proposed. There are at least two distinct quaternary conformations in equilibrium with each other which correspond to the liganded and unliganded state. The former state has fewer bonds between subunits and thus can develop full oxygen affinity, whereas unliganded haemoglobin has strong bonds between the subunits and thus has a lower oxygen affinity.

There is little information about the dynamics of the structural changes accompanying ligand binding. However, the most recent theory which attempts to explain these conformational changes suggests that steric strain, arising as the liganded ferrous ion attempts to move to its optimum position in the haemoglobin molecule, decreases the stability of the deoxy quaternary structure, so that it changes to the liganded quaternary structure, and induces tertiary structure changes, which impose no restraints to subsequent ligand binding

(Baldwin and Chothia, 1979).

It is this co-operative binding of oxygen by haemoglobin which produces the characteristic sigmoid shape of the oxygen dissociation curve.

If the low affinity deoxy-structure is stabilized, or destabilized relative to the oxy-structure, the overall oxygen affinity will be decreased or increased. It has become clear over the last decade that most changes in the oxygen affinity of normal haemoglobin can be explained solely by changes in the stability of the deoxy-conformation, the oxy-conformation remaining unaltered.

This is true of the Bohr effect, whereby hydrogen ions increase the stability of the deoxy-conformation relative to the oxy-conformation. Much work involving x-ray crystallography, as well as chemical modifications, has shown that a number of groups in the haemoglobin molecule act as weak bases. In the charged state, these groups, which include the imino group of the C-terminalhistidine of the β-chains, the amino group of the N-terminal valine of the α-chains, and possibly the imino group of one of the histidines of the α-chains, participate in the formation of salt bridges which stabilize the deoxy conformation. As hydrogen ion concentration decreases, the groups lose some of their charge, reducing salt bridge formation, and decreasing the stability of the deoxy-conformation, allowing the oxygen affinity to increase.

Perutz also postulated that it was the breaking of the salt bridges on oxygenation which causes a reduction in the pK of these weak bases, with the resultant release of protons, characteristic of the Haldane effect.

2,3-DPG lowers oxygen affinity by a similar mechanism of binding to the positively charged groups in the deoxy-haemoglobin molecule and stabilizing the conformation. Chemical and crystallographic analysis identified the 2,3-DPG binding site as the entrance to the central cavity which involves the N-terminal α amino groups, histidines (2) and (143) and one of the lysines (82) of the β chains. The binding of 2,3-DPG to these groups not only stabilizes the deoxy-conformation, but also decreases the oxygen affinity of the deoxy-conformation, producing a two conformational, three affinity-state system. On oxygenation, the sides of the central cavity converge and the 2,3-DPG molecule is expelled.

The binding of 2,3-DPG to haemoglobin is pH-dependent, increasing as the pH falls, and it is now known that 2,3-DPG binds to oxyhaemoglobin as well as deoxyhaemoglobin, although the affinity of 2,3-DPG for oxyhaemoglobin is only about half of that for deoxyhaemoglobin.

Carbon dioxide has the ability to alter the oxygen affinity of haemoglobin, not only by its effect on pH, but also by reacting reversibly with the N-terminal α amino groups of the α and β subunits to form carbamate compounds. Although carbamate formation occurs at all four N-terminal α amino groups, those of the β subunits have a three-fold higher affinity for carbon dioxide than those of the α subunits, as the latter are primarily protonated in deoxyhaemoglobin, and carbon dioxide will only react with uncharged groups.

The structural explanation for the effect of carbon dioxide on the oxygen affinity is believed to be a salt bridge formed between the negatively charged carbamate group and a positively charged group in the deoxyhaemoglobin molecule,

probably the ε-amino group of a β-chain lysine. On oxygenation these interactions are disrupted and the pK of the carbamate groups is raised, causing the carbamate to dissociate, and explains why deoxyhaemoglobin contains more carbon dioxide at any given carbon dioxide tension than oxyhaemoglobin.

Carbon monoxide, unlike the other ligands of haemoglobin mentioned above, combines reversibly with the ferrous ion in the haem group, with the result that oxygen transport is impaired by two mechanisms: carbon monoxide lowers the oxygen carrying capacity of the blood, and increases the oxygen affinity of the remaining haemoglobin, resulting in impaired release of oxygen to the tissues.

Since the affinity of haemoglobin for carbon monoxide is approximately 200-250 times its affinity for oxygen, exposure to low carbon monoxide concentrations can result in clinically significant impairment in oxygen transport.

All the ligands mentioned above are involved in complex interactions which determine the oxygen affinity of haemoglobin (fig 8). In view of the fact that carbon dioxide and 2,3-DPG combine with the N-terminal amino groups of the β chains, a competition between the two ligands is to be expected. As a result of this competition, the contribution of carbamate to the in vivo transport of carbon dioxide in the blood is lower than originally calculated, and only 10.5% of carbon dioxide is carried as carbamate. Similarly, carbon dioxide at a partial pressure of 40 mm Hg reduces 2,3-DPG binding by 25%. Thus the combined effect of carbon dioxide and 2,3-DPG on the oxygen affinity will not simply be the sum of their individual effects.

As the binding of 2,3-DPG and carbon dioxide to haemoglobin is pH-dependent, they both influence the Bohr effect. The fixed

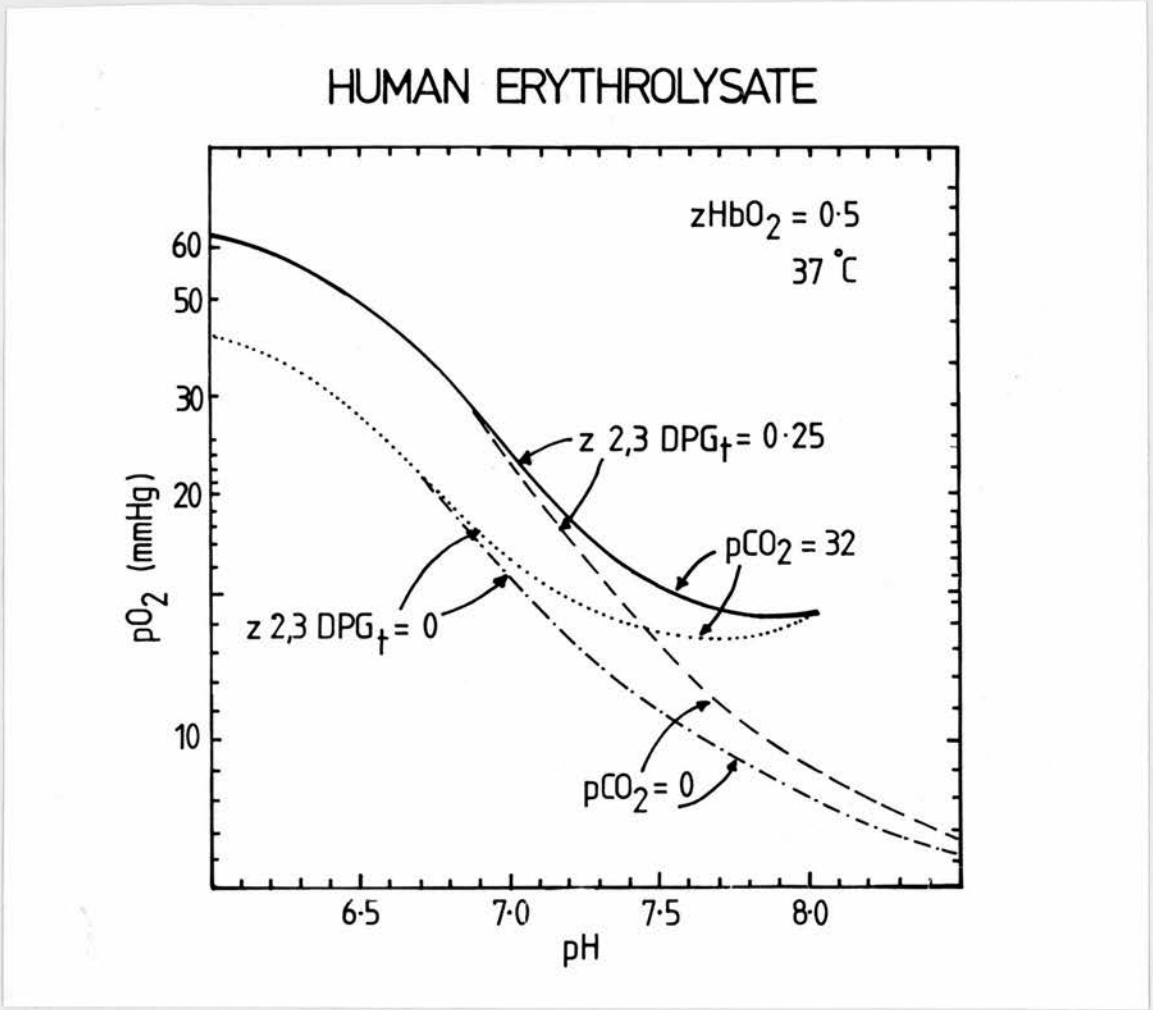


Figure 8

The composite effects of CO_2, 2,3-DPG, and pH in human erythrolysate on the oxygen affinity of haemoglobin. (Rorth, 1972).

acid Bohr effect is lower in the presence of carbon dioxide than in its absence, an effect due to carbamino-bound carbon dioxide exerting its own influence on the oxygen dissociation curve. Similarly, the Haldane effect is reduced in the presence of carbon dioxide because on oxygenation the dissociation of carbamate compounds removes H^+ ions and reduces the change in H^+ caused by oxygenation.

At physiological pH, 2,3-DPG increases the fixed acid Bohr effect, which appears to be caused by suppression of carbamate formation. However, 2,3-DPG has the opposite effect on the carbon dioxide Bohr effect, which is reduced in the presence of 2,3-DPG. This effect is again due to suppression of carbamate formation, with the result that at normal 2,3-DPG levels, the CO_2 Bohr effect is primarily a direct effect of H^+ ions.

The Haldane effect is increased by 2,3-DPG due to an increase in the pK values of the weak bases when 2,3-DPG binds to haemoglobin.

The quantitative contribution of protons, carbon dioxide and 2,3-DPG towards the oxygen affinity will depend on the pH. On the acid side of physiological pH, 2,3-DPG and proton binding will predominate, as 2,3-DPG binds to charged groups and increases the Bohr effect. On the alkaline side of physiological pH, carbon dioxide binding will predominate as it binds mainly to uncharged amino groups and decreases the Bohr effect, so that changes in proton or 2,3-DPG concentration will have a smaller effect on the oxygen affinity.

The situation in vivo is further complicated as the concentration of 2,3-DPG, as well as its binding constant, varies with pH. Thus, in acute respiratory acidosis, with a

rise in PCO_2, the oxygen dissociation curve will be displaced to the right by the Bohr effect, and to some degree, by greater carbamate formation at the higher PCO_2, and also by increased 2,3-DPG binding to haemoglobin. However, after several hours, 2,3-DPG concentration will fall, and the dissociation curve will be displaced to the left by reduced binding of 2,3-DPG to haemoglobin, and also by the rise in intracellular pH which will accompany the reduction in 2,3-DPG concentration. The in vivo consequence is that the oxygen dissociation curve remains fairly constant in established respiratory failure, although the in vitro $P50_{(7.4)}$ will fall.

In metabolic acidosis, where carbon dioxide tension remains constant, carbamate formation will not increase as in respiratory acidosis, and may, in fact, fall, with the effect that the initial rightward shift in the oxygen dissociation curve would not be as great as in respiratory acidosis.

In respiratory alkalosis the Bohr effect, together with a decrease in 2,3-DPG binding and carbamate formation will contribute to the leftward displacement of the oxygen dissociation curve. However, the subsequent rise in 2,3-DPG concentration, in response to the alkaline pH, will shift the curve back to the right with the result that, in vivo, the P50 is normal.

Again, in metabolic alkalosis, at a constant carbon dioxide tension, the changes outlined above will occur, but carbamate formation will increase and antagonise the leftward shift in the oxygen dissociation curve.

In hypoxaemia due to chronic bronchitis, complicated by hypercapnia, the above mechanisms will operate to maintain an adequate oxygen supply to the tissues, but further compensatory mechanisms may also operate, such as increased red cell production

and 2,3-DPG concentrations, but changes and adaptations amongst these patients are by no means uniform.

CHAPTER 2

Aims of the Present Study

The clinical benefit of oxygen administration to selected patients with chronic obstructive pulmonary disease has been recognised for many years. Various groups have found oxygen therapy can control congestive right heart failure and secondary polycythaemia, and improve exercise tolerance and pulmonary artery pressure. However, the effect of long term oxygen treatment on the position and shape of the oxygen dissociation curve has not been determined, and as oxygen transport to the tissues is dependent upon the oxygen dissociation curve, together with haemoglobin concentration, arterial oxygen tension and cardiac output, this effect, if one exists, is important in ascertaining the physiological benefits of long term oxygen therapy.

Studies carried out by Sagone, Lawrence and Balcerzak (1973) and Smith and Landaw (1978) suggested that elevated carboxy-haemoglobin levels common in smokers, resulted in tissue hypoxia with adaptive increases in red cell mass, which could be reversed when smoking was discontinued. Many patients with chronic pulmonary disease continue to smoke and have elevated carboxy-haemoglobin concentrations which will increase existing tissue hypoxia and secondary polycythaemia. Does a relationship exist between the degree of secondary polycythaemia in patients with severe chronic bronchitis, and their smoking habits, and can this be explained by impairment of oxygen transport to possible sites within the renal glomerulus or medulla where erythrogenin is released?

In normal healthy subjects exercise capacity has been shown to decrease when carboxyhaemoglobin levels are elevated,

and impairment of maximal oxygen uptake has been shown to occur at carboxyhaemoglobin levels as low as 4.3%. As carboxyhaemoglobin levels of 5 - 15% are often encountered in patients with chronic bronchitis whose exercise tolerance is already impaired by hypoxaemia, do such carboxyhaemoglobin levels constitute a significant further impairment of oxygen transport during exercise?

In many patients, chronic obstructive lung disease is complicated by chronic carbon dioxide retention, but the exact mechanism of this retention is still not clear. The removal of an hypoxic stimulus to ventilation by administration of high concentrations of oxygen may lead to carbon dioxide retention, but the classical studies of Christianson, Douglas and Haldane (1914) showed that, in normal subjects, oxygenation of haemoglobin at a constant carbon dioxide content also increases the carbon dioxide tension. The magnitude of this effect can be calculated by the equation of Visser (1960):-

$$\frac{CbCO_2}{CpCO_2} = 1 - \frac{0.0215 \times O_2 \text{ capacity}}{(2.244 - 0.422\ SO_2)(8.74 - pH)}$$

where $CbCO_2$ = whole blood CO_2 content

$CpCO_2$ = plasma CO_2 content

SO_2 = O_2 saturation/100

and O_2 capacity is expressed as vol.per vol. of blood

his equation was derived from observations of Van Slyke and Sendroy 1928), made using normal human blood and horse blood, not that f hypoxic hypercapnic human subjects with chronic bronchitis nd emphysema. Therefore, as the C.D.H. effect may contribute o the hypercapnia seen in these patients, determination of the agnitude of this effect is required.

The purpose of this study, therefore, was to answer the following questions:-

1. Does long term oxygen therapy affect oxygen transport by changing the oxygen dissociation curve of blood and other haematologic variables?
2. Can the degree of secondary polycythaemia in patients with chronic obstructive lung disease be related to their smoking habits and degree of tissue hypoxia?
3. Does acute elevation of carboxyhaemoglobin concentration affect the exercise tolerance of patients with chronic bronchitis and can this be related to tissue oxygen supply?
4. Does whole blood CO_2 content, calculated using the equation of Visser (1960), compare with the CO_2 content measured in the infra red gas analyser (I.R.G.A.) in normal subjects and patients with chronic obstructive pulmonary disease, and can this equation accurately predict the magnitude of the C.D.H. effect?
5. Is the C.D.H. effect in hypoxic, hypercapnic subjects similar to that in normal subjects, and to what extent could this mechanism contribute to the hypercapnia present in some patients with chronic bronchitis?
6. Is the C.D.H. effect altered by elevated carboxy-haemoglobin concentrations, present in many of these patients who are cigarette smokers?

During the course of the study, marked variations in the .D.H. effect in normal women posed the following additional uestions:-

7. Does the C.D.H. effect vary through the normal menstrual cycle?

8. What are the biochemical mechanisms of such changes in the C.D.H. effect?

CHAPTER 3

METHODS

1. Instrumentation

(a) Blood Gas Analysis

All pH and blood gas data were obtained using the IL model 313 automatic pH/Blood Gas Analyser. Whole blood PO_2, PCO_2 and pH values can be determined simultaneously, and less than 0.4 ml blood was required for all three measurements.

All three electrode systems were calibrated each morning. The pH electrode was calibrated using B.D.H. buffer solutions with pH values of 6.845 $\pm$ 0.005 and 7.385 $\pm$ 0.005 at 38°C, and the calibration was checked at least once during the day. The PO_2 and PCO_2 electrodes were calibrated with two known gas mixtures, analysed using the Lloyd Gas Analyser (Gallenkamp), duplicate determinations of gas samples agreeing to within 0.03%. One mixture of approximately 5% CO_2, 12% O_2, balance N_2, was used to set the upper point of the oxygen electrode and the lower point of the carbon dioxide electrode, while the other gas mixture, which consisted of approximately 10% CO_2, balance N_2, was used to determine the slope of the oxygen and carbon dioxide electrodes. This two-point calibration procedure was repeated at least once during the day, but a single-point calibration check was carried out between each sample.

Each day the accuracy of the blood gas analyser was checked by tonometry of an aliquot of whole blood with a known gas mixture, containing approximately 7% O_2, 7% CO_2, balance N_2, accurately determined with the Lloyd Gas Analyser. In this way the PO_2 and PCO_2 of the blood sample became known, and when introduced into the blood gas analyser, could reveal faults

in electrode response, calibration, temperature or sample introduction.

The precision of each electrode system was assessed from 21 consecutive determinations of the same blood sample. The mean values ± 1 standard deviation of these 21 aliquots were: pH 7.30 ± 0.008; PCO_2 53.9 ± 0.53 mm Hg; and PO_2 43.5 ± 0.37 mm Hg; indicating a greater degree of precision than that obtained by Weisbrot, Kambli and Gorton (1974) using the same instrument.

(b) Tonometer

An IL 237 tonometer was used for in vitro equilibration of blood samples for all research and quality control procedures. This tonometer is a thin film, open system tonometer consisting of a thermostatically controlled circulating water bath containing a gas humidifyer and removable cuvette. By means of intermittent rotation of the cuvette, the blood is spread in a thin film and brought into close contact with a stream of humidified gas. All samples were equilibrated at 37°C with gas at a flow rate of 350 mls/min for 20 minutes, to ensure complete equilibration, with minimal haemolysis (Chalmers, Bird and Whitwam, 1974). Comparison of plasma haemoglobin levels before and after tonometry of a sample for 20 minutes showed no change, indicating haemolysis was not induced by tonometry.

c) Co-oximeter

Oxyhaemoglobin and carboxyhaemoglobin saturations, and haemoglobin concentrations were estimated using the IL 182 co-oximeter. Whole blood is introduced into the apparatus direct from the syringe and is automatically haemolysed, mixed,

thermostated and pumped to the cuvette. The instrument measures the absorbance of the sample at 548 nm, 568 nm and 578 nm. where two or more of the haemoglobin derivatives have equal extinction coefficients. The computational matrix then applies the Beer-Lambert equation to the data, and solves three simultaneous equations and finally gives a direct read out of haemoglobin concentration as g/100 ml, and oxy- and carboxy-haemoglobin as percentage saturation according to the following equations:-

$$[Hb]\ \text{total} = [Hbr] + [HbO_2] + [COHb]$$

$$COHb\ \% = \frac{[COHb]}{[Hbr] + [HbO2] + [COHb]} \times 100$$

$$HbO_2\ \% = \frac{[HbO2]}{[Hbr] + [HbO_2] + [COHb]} \times 100$$

where $[Hbr]$ = concentration of reduced haemoglobin

$[HbO_2]$ = concentration of oxyhaemoglobin

$[COHb]$ = concentration of carboxyhaemoglobin

The important aspects of these computations is that oxygen saturation is expressed as the percentage saturation of total haemoglobin with oxygen, as distinct from the percentage saturation of available haemoglobin with oxygen, which does not take account of carboxyhaemoglobin concentration.

For calibration of the IL 182 co-oximeter, freshly drawn heparinised blood from a non-smoker was used, to ensure low carboxyhaemoglobin levels. Usually blood from a female member of staff was used as the haemoglobin concentration in females is close to the mid range of expected haemoglobin values.

The haemoglobin concentration of the sample was accurately determined by the cyanomethaemoglobin method, and 3 x 10 ml aliquots of blood were equilibrated with O_2, CO and N_2 respectively, and the calibration procedure carried out according to the manufacturer's instructions. Calibration was necessary once in 5 - 6 months, but weekly checks were made by comparing haemoglobin values determined by the cyanomethaemoglobin method with those determined by the co-oximeter.

The mean value $\pm$ 1 standard deviation for 21 consecutive readings of the same sample on the oxyhaemoglobin saturation channel was 67.1 $\pm$ 0.34%; on the carboxyhaemoglobin saturation channel 8.5 $\pm$ 0.28%; and on the haemoglobin concentration channel 15.0 $\pm$ 0.09 g/100 ml. These standard deviations compare closely with those of Maas, Hamelink and Leeuw (1970) for the same instrument.

(d) Lexington Lex-O_2-Con

All oxygen contents were measured by the Lexington Lex-O_2-Con apparatus, which measures total oxygen content from a 20 µl sample of whole blood. The oxygen evolved from the blood combines with a fuel cell which releases a stream of electrons. These electrons form a current which is propotional to the amount of oxygen present and the instrument gives a direct digital readout of oxygen content in mls per 100 mls blood.

The fuel cell was checked periodically by comparing oxygen content results from the Lex-O_2-Con with those from the Van Slyke manometric technique, and if necessary the fuel cell was recharged. Calibration checks were also made daily by comparing the measured oxygen content of a 20 µl sample of room air with the calculated value, taking into account the

temperature, relative humidity and barometric pressure.

Twenty-one measurements of the same blood sample gave a mean value ± 1 standard deviation of 14.6 ± 0.38 mls 0_2/100 ml blood.

(e) Spectrophotometer

The Unicam SP 1800 Ultraviolet Spectrophotometer was used for determination of 2,3-diphosphoglycerate concentrations. This spectrophotometer is a manually operated double beam grating instrument, which measures the logarithmic ratio of reference and sample beam light intensities, and displays the absorbance measurements on a meter with a linear absorbance scale.

The photometric accuracy determined by the manufacturer was ± 1% of full scale on all ranges, and the reproducibility was also 1% of full scale values on all ranges.

(f) Infra-red Gas Analyser

Whole blood carbon dioxide content was measured by a new method, modified from that described by Van Kempen and Kreuzer (1972). An infra-red gas analyser (Grubb Parson Model IRGA 20), with a range of 0 - 0.5% CO_2 was used after modifications had been made. Most of the internal tubing was replaced by copper tubing to reduce diffusion of carbon dioxide, and a fan-assisted air circulation system, together with a proportional temperature controlling system were installed to maintain the internal temperature at 37°C as the instrument was found to be sensitive to temperature variations.

A block diagram of the apparatus used to measure whole blood carbon dioxide content is shown in Fig. 9. Room air, drawn in through soda lime, was pumped at a rate of 50 litres/hour through the apparatus and then exhausted to air, to flush all carbon dioxide from the system and zero the instrument. The circuit was then closed at the two 3-way stopcocks and 0.25 ml

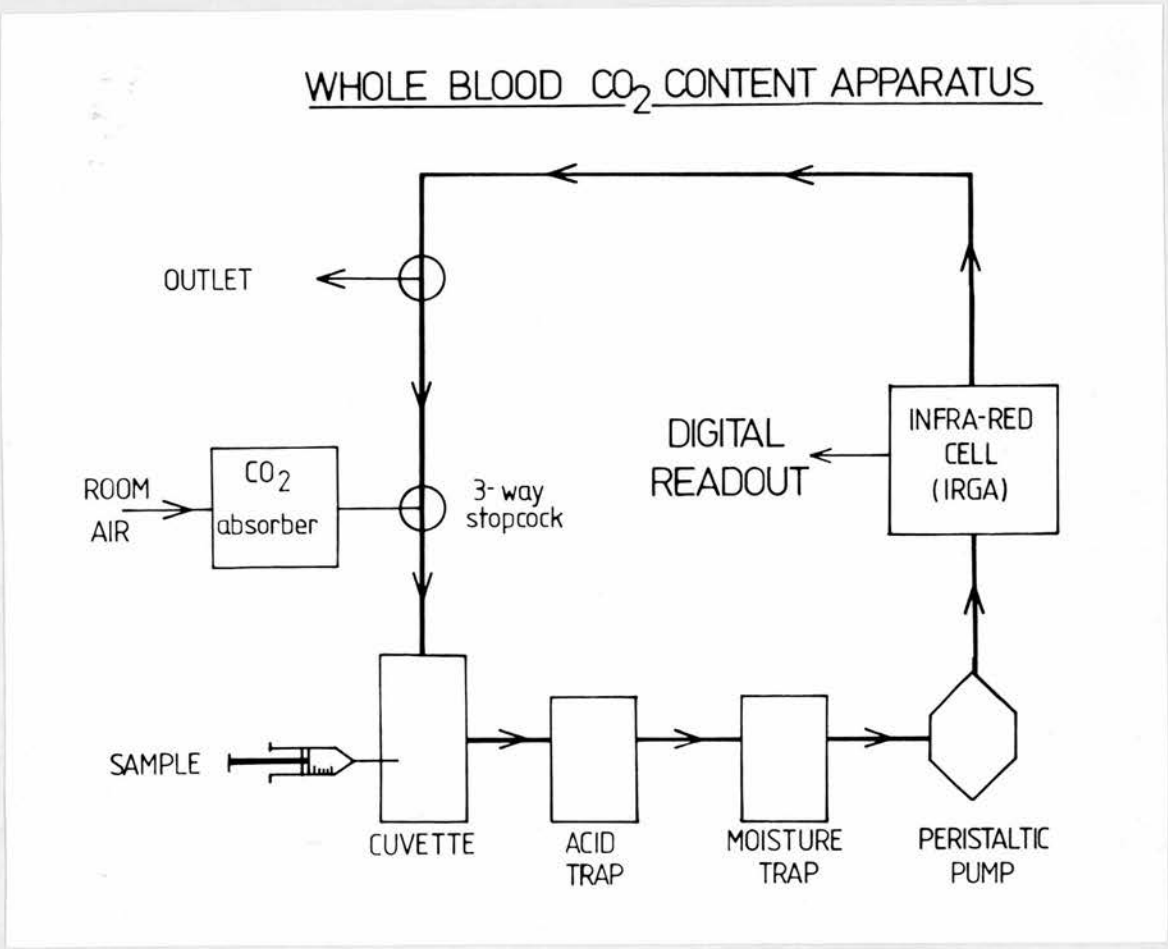


Figure 9

Schematic representation of apparatus used to estimate whole blood CO_2 content by the infra-red gas analyser (I.R.G.A.).

of whole blood injected into the cuvette containing 4.0 ml of 0.1 mol/l lactic acid and an antifoam agent. The carbon dioxide in the sample was extracted by the lactic acid and constantly recirculated through the apparatus until a steady reading was obtained. Results from the infra-red gas analyser were relayed through a Solartron A220 digital voltmeter to a Teletype printer which printed readings at 20 second intervals. When readings had been constant for two minutes, the result was accepted, and the cuvette was cleaned and prepared for the next sample, and the circuit opened to flush carbon dioxide from the system. Carbon dioxide concentrations were read from a calibration graph, drawn each day, using standard sodium carbonate solutions of approximately 20, 30 and 50 mmol CO_2/litre concentration, determined accurately by the Van Slyke manometric technique.

Calibration of the infra-red gas analyser was checked periodically by circulating 0.4% CO_2 in N_2, prepared by the Wosthoff gas mixing pumps, through the instrument, and adjusting the gain control until the meter reading gave the expected value. Various concentrations of CO_2 in N_2, ranging from 0 - 0.5% were then circulated through the instrument to check linearity of the detector.

To assess the accuracy of CO_2 concentrations determined by this instrument, 51 samples of blood with carbon dioxide concentrations ranging from 13 - 35 mmol/litre were analysed using both the infra-red gas analyser and the Van Slyke manometric apparatus. In addition, precision was assessed by carrying out 14 repeated estimations on samples of blood containing approximately 14, 25 and 33 mmol CO_2/litre.

Assessment of Infra-red Gas Analyser (I.R.G.A.) Performance

The I.R.G.A. detector reading was a linear function of CO_2 concentration for values greater than 0.16% CO_2 (Fig.10). By minimising the closed circuit volume of the instrument, the CO_2 extracted from the whole blood samples was sufficient in all cases to bring the CO_2 concentration within the instrument into the linear range of the detector.

The CO_2 concentrations determined in 51 samples of whole blood by the I.R.G.A. and the Van Slyke manometric technique are compared in Figure 11. The linear regression equation: $[CO_2]_{(I.R.G.A.)} = 0.99 [CO_2]_{(Van\ Slyke)} + 0.286$, $n = 51$, $r = 0.999$, $P < 0.001$, describes the relationship, with 95% confidence limits for the population of $\pm$ 0.32 mmol/l. There was no significant difference between this line and the line of identity.

Tests of reproducibility of 14 replicate samples at three different levels of CO_2 gave a standard deviation of 0.13 mmol/l with a mean of 13.9 mmol/l, a standard deviation of 0.2 mmol/l with a mean of 23.6 mmol/l, and a standard deviation of 0.22 mmol/l with a mean of 33.4 mmol/l, yielding coefficients of variation of 0.9%, 8% and 0.7% respectively.

No cross-sensitivity was detected with the other gases normally present in blood, including carbon monoxide, present in the blood of cigarette smokers.

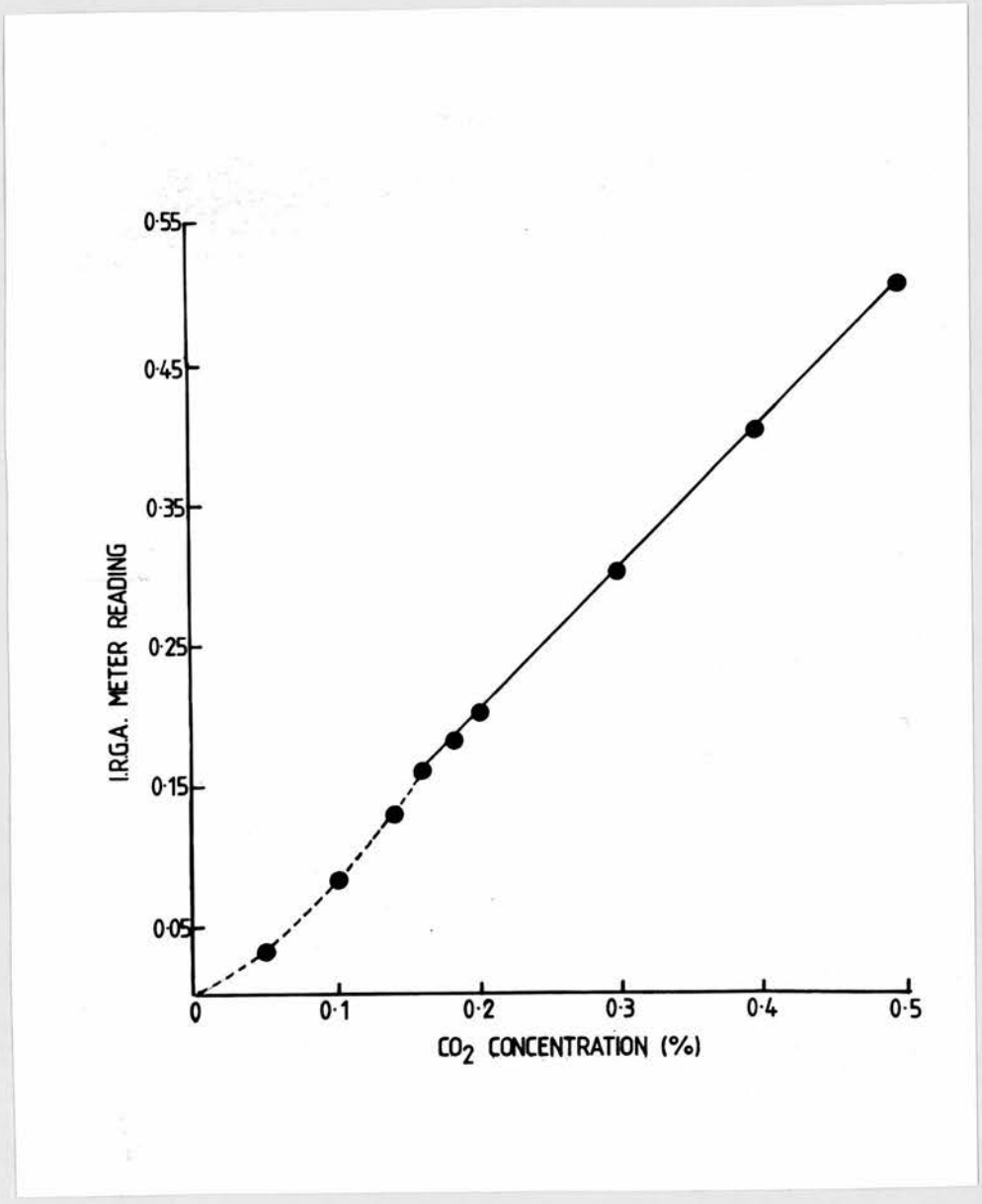


Figure 10

The relationship between I.R.G.A. meter reading and CO_2 concentration. The relationship is linear between CO_2 concentrations of 0.16 - 0.50%, and in all determinations of whole blood CO_2 content, the CO_2 concentration of the circulating gas was within this range.

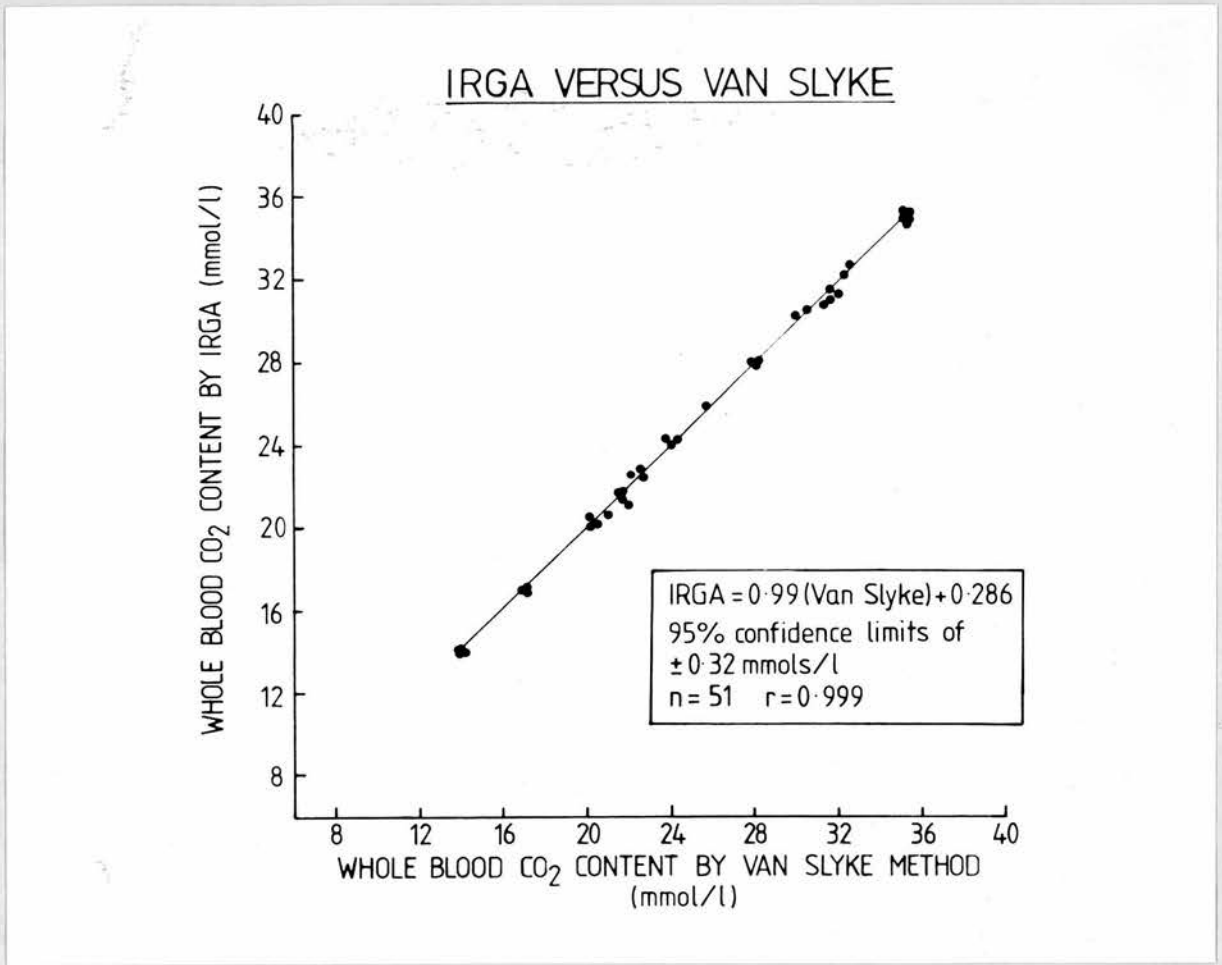


Figure 11

A comparison of whole blood CO_2 content estimations measured using the I.R.G.A. and the Van Slyke manometric apparatus ($r = 0.999$, $P < 0.001$).

Discussion of the method developed for the determination of whole blood C02 content

The I.R.G.A. method provides a simple means of measuring whole blood CO_2 content. Results appertaining to the assessment of this method show that it is capable of measuring whole blood CO_2 content in the range 14 - 35 mmol/l blood with an accuracy comparable to that of the Van Slyke manometric technique, and with a similar degree of precision.

The I.R.G.A. method has additional advantages over the Van Slyke manometric technique, insomuch as it is not dependent upon a high degree of operator skill, requiring only the accurate delivery of the 0.25 ml whole blood sample, which is facilitated by the use of an accurately graduated glass syringe fitted with a Chaney adaptor. After the introduction of the whole blood sample, the analysis will proceed unassisted, the instrument read-out being recorded by the teletype printer, every 20 seconds.

The analysis time of the IRGA method is marginally faster than that of the Van Slyke manometric technique, analysing one sample every 15 minutes, whereas in our laboratory, the Van Slyke manometric technique requires 15 - 20 minutes to complete an analysis and to prepare the instrument for the next sample, the operator's presence being necessary throughout.

Therefore, as the performance of the IRGA compares favourably with that of the Van Slyke manometric technique, and also provides greater ease of operation, we feel that this method of estimating whole blood CO_2 content is a marked improvement over the technique of Van Slyke.

2. Techniques

(a) Determination of P50

Venous blood was sampled into a 50 ml heparinised syringe containing a sterile, stainless steel washer which ensured complete mixing of the sample. Three 5 ml aliquots of blood were equilibrated in the IL 237 tonometer with three gas mixtures containing approximately 5% CO_2, three different O_2 concentrations which yielded oxygen saturations between 30 - 70%, and balance N_2. At the end of the equilibration period, the sample was drawn anaerobically into a syringe containing a mixing washer, and the oxygen and carboxy-haemoglobin saturations, and the haemoglobin concentration were determined by the IL 182 co-oximeter. Oxygen content determinations were made using the Lex-O_2-Con instrument and finally blood gas tensions and pH were determined in the IL 313 Blood Gas Analyser.

The PO_2 values for each sample were corrected to pH 7.40 and base excess zero using the formula of Severinghaus (1966) which describes the Bohr effect quantitatively:-

$$\Delta \log PO_2 = 0.0013 \text{ Base Excess } -0.48 \ \Delta pH$$

By plotting oxygen saturation against the corrected PO_2 values, P50 at pH 7.4 and base excess 0 was determined. The P50 was also determined by the Hill plot of $\log \frac{(O_2 \text{ saturation})}{(100 - O_2 \text{ saturation})}$ against $\log PO_2$ (corrected to pH 7.4 and base excess 0), and the slope of the resulting straight line gave Hill's "n" a measure of haem-haem interaction.

All measurements for determination of P50 were carried out within two hours of withdrawal of the sample to minimise in vitro changes in P50 (Aberman, Cavanilles, Michaels, Shubin and Weil, 1976).

(b) Determination of Intra-erythrocyctic pH

The red cell pH of arterial and venous samples was determined by the freeze-thaw technique of Hilpert, Fleischmann, Kempe and Bartels (1963). Blood was drawn anaerobically into a 2 ml syringe and centrifuged at 3000 rpm for 15 minutes at $4^{o}C$. The plasma was displaced and the remaining red blood cells were stored in dry ice ($-80^{0}C$) for 30 minutes then thawed at $37^{o}C$. The pH was then determined as for whole blood.

(c) Determination of Packed Cell Volume

The packed cell volume was determined by the micro-haematocrit technique using the Hawksley microhaematocrit centrifuge.

(d) Determination of the Christiansen, Douglas, Haldane (CDH) Effect

A 35 ml sample of venous blood was drawn into a heparinised syringe containing a stainless steel washer to ensure adequate mixing of the blood. Eight 4 ml aliquots of blood were equilibrated in the IL 237 tonometer with various concentrations of $C0_2$ in N_2, which, when mixed with air or 0_2 in the Wosthoff gas mixing pumps, gave $PC0_2$ values of approximately 20, 40, 50 and 70 mm Hg at two $P0_2$ levels designed to produce blood oxygen saturations of 50 and 100%.

When equilibration was complete, the samples were drawn anaerobically into a syringe and measurements of total carbon dioxide content, oxygen and carbon dioxide tensions and pH were made. Oxy- and carboxyhaemoglobin saturations, haemoglobin concentration and the oxygen content of each sample were also determined.

By plotting the carbon dioxide content of each sample against the carbon dioxide tension, carbon dioxide dissociation curves were drawn for oxyhaemoglobin saturations of 50 and 100%. The CDH effect, expressed as the change in carbon dioxide content at a PCO_2 of 40 mm Hg as fully oxygenated blood is reduced to 50% oxygen saturation, was then obtained as the difference in carbon dioxide content between the two curves at PCO_2 40 mm Hg (Fig. 29).

(e) Determination of 2,3-Diphosphoglycerate (2,3-DPG) Concentration

2,3-DPG concentration was estimated by the ultraviolet method of Sigma (Technical Bulletin 35 -UV). This method of analysis involves the enzymatic hydrolysis of 2,3-DPG to 3-phosphoglycerate (3-PGA), catalysed by 2,3-DPG phosphatase, which is present in purified preparations of phosphoglycerate mutase, and requires phosphoglycolic acid as a stimulator.

$$\text{2,3-DPG} \xrightarrow[\text{Phosphoglycolic Acid}]{\text{2,3-DPG Phosphatase}} \text{3-PGA + inorganic phosphate}$$

The 3-PGA is then coupled with phosphoglycerate kinase and glyceraldehyde phosphate dehydrogenase to form 3-phospho-glyceraldehyde.

$$\text{3-PGA + ATP} \xrightarrow{\text{phosphoglycerate kinase}} \text{1,3-DPG + ADP}$$

$$\text{1,3-DPG + NADH} \xrightarrow[\text{dehydrogenase}]{\text{glyceraldehyde phosphate}} \text{3-phosphoglyceraldehyde + NAD}$$

The decrease in NADH is proportional to the amount of 2,3-DPG present initially and by measuring the change in optical density of the mixture as NADH is oxidised to NAD, the concentration of 2,3-DPG can be calculated.

Reagents

1. Trichloroacetic acid : 8% w/v solution in distilled water. Stored at 0 - 5°C.
2. NADH buffer solution : 1 mg NADH dissolved in 8.0 ml triethanolamine buffer solution. Stable for a few hours only and stored at 0 - 5°C when not in use.
3. ATP : 500 mg adenosine 5-triphosphate dissolved in 5 ml triethanolamine buffer. Stable for several months when stored frozen.
4. Glyceraldehyde phosphate dehydrogenase/phosphoglycerate kinase mixture (GAPD/PGK) from Sigma and stored at 0 - 5°C.
5. Phosphoglycerate mutase enzyme : obtained from Sigma and stored at 0 - 5°C.
6. Phosphoglycolic acid : 50 mg dissolved in 5 ml distilled water and stored frozen.

Protein-free Filtrate

A protein-free filtrate of whole blood was prepared by addition of 1 ml of whole blood to 3.0 ml cold trichloroacetic acid. After thorough mixing, it was centrifuged at 3000 rpm for 15 minutes at 4°C and the supernatant decanted

off and stored frozen until analysed.

Analysis

Blank and test cuvettes were set up as shown below:-

Reagent	Blank	Test
NADH buffer solution	1 ml	1 ml
ATP solution	0.04 ml	0.04 ml
Water	0.1 ml	-
Protein-free filtrate	-	0.1 ml
GAPD/PGK mixture	0.01 ml	0.01 ml
Phosphoglycerate mixture	0.01 ml	0.01 ml

After thorough mixing the optical density at 340 nm was measured by the Unicam SP 1800 spectrophotometer. 0.04 ml phosphoglycolic acid was added to each cuvette to start the reaction and the optical density measured after 30 minutes.

Calculation

Any change in optical density of the blank was subtracted from the change in the test cuvette, and 2,3-DPG concentration calculated by:-

$$[2,3\text{-DPG}] = \frac{\Delta O.D. \times (\text{volume in cuvette})}{(\text{mMolar extinction coefficient})} \times (\text{volume of original sample})$$

$$= \frac{\Delta O.D. \times 1.2}{6.22 \times 0.025}$$

This gives 2,3-DPG concentration in μmoles/ml whole blood, which is then corrected to μmoles/g haemoglobin.

The accuracy of this method of determination was assessed by recovery experiments, in which percentage recovery ranged

from 83 - 100%, with a mean of 90%. This result was accepted, as the 2,3-DPG salt used in the tests was not the most pure, so a larger error was to be expected. The mean $\pm$ 1 standard deviation of 10 determinations on the same sample was 2.41 $\pm$ 0.02 µmoles 2,3-DPG/ml blood (P. Tweeddale, Personal Communication).

CHAPTER 4

Study of Long Term Oxygen Therapy

Subjects and experimental design

The patients (11 women, 15 men) were aged between 42 - 68 years at the start of the study, and all suffered from hypoxic cor pulmonale secondary to chronic bronchitis and emphysema, diagnosed from the history, clinical examination, chest x-ray and the presence of severe airways obstruction ($FEV_{1.0}$ 0.20 - 1.35, mean 0.58 $\pm$ 0.23). At the start of the study all were hypoxaemic (arterial PO_2 44 - 60 mm Hg) and hypercapnic (arterial PCO_2 46 - 67 mm Hg) at rest, breathing air, and had had one or more documented episodes of cor pulmonale. The patients were clinically stable and without significant change in arterial blood gases, $FEV_{1.0}$ or body weight for three weeks prior to the first assessment. This assessment included measurement of $P50_{(7.4)}$, Hill's 'n', oxygen capacity, 2,3-DPG concentration, carboxyhaemoglobin saturation, haemoglobin concentration, packed cell volume, arterial and venous pH of whole blood and red cells, and arterial blood gases.

On entry to the study, the patients were randomised; 13 were designated as control subjects who would receive no long term oxygen therapy and 13 were designated as treated subjects who would receive long term oxygen therapy for 15 hours per day. Controlled oxygen therapy was provided through nasal prongs at a flow rate of 2 L/minute, designed to raise the arterial oxygen tension to over 60 mm Hg (Leggett, Cooke, Clancy, Leitch, Kirby and Flenley, 1976).

The progress of the patients was followed at routine clinic visits and at assessment after 12, 24 and 36 months of the study, when all the above measurements were repeated.

Statistical comparisons of mean values between the two groups of patients were carried out using unpaired 't' test, and comparisons within subjects by paired 't' test.

:esults appertaining to the study of long term domiciliary oxygen herapy

There was no significant difference in age, severity of irways obstruction, red cell mass, or mean pulmonary artery pressure t the start of the study, between those subjects designated as ontrol patients, and those who were to receive long term oxygen herapy (Table 1a,b). In addition, there was no significant difference n average values of arterial blood gases, extracellular and intra-ellular pH, haemoglobin concentration, packed cell volume (P.C.V.), mean orpuscular haemoglobin concentration (M.C.H.C.), in vitro $P50_{(7.4)}$, ill's n, 2,3-DPG concentration and carboxyhaemoglobin levels etween the two groups at the start of the study, nor after one year therapy (Table 2). However, after two years of the study, the ean haemoglobin concentration and packed cell volume in the patients ceiving oxygen therapy (13.6 $\pm$ 1.4 g/dl and 45.0 $\pm$ 4.8 ml/dl spectively) were significantly lower ($P < 0.05$) than those of the ntrol group patients (mean haemoglobin concentration 16.0 $\pm$ 1.9 g/dl d P.C.V. 52.0 $\pm$ 5.2 ml/dl), this trend continuing at the third year sessment, when those patients receiving oxygen therapy again monstrated a lower mean haemoglobin concentration (13.1 $\pm$ 1.6 g/dl) d packed cell volume (44 $\pm$ 5.3 ml/dl) than the control group ients (mean haemoglobin concentration 17.4 $\pm$ 0.4 g/dl, mean .V. 57 $\pm$ 1.0 ml/dl). ($P<0.01$, $P<0.02$ respectively.)

The average $P50_{(7.4)}$ value determined in the patients eiving long term oxygen therapy did not differ significantly from t in the control group patients until the third year of the study, n the mean $P50_{(7.4)}$ in those patients receiving oxygen therapy .3 $\pm$ 2.1 mm Hg) was significantly lower ($P < 0.05$) than that in the surviving control group patients (30.1 $\pm$ 0.8 mm Hg), but there no significant difference in the shape of the oxygen dissociation

curve, indicated by the average value of Hill's n, between the two groups, at any time during the study.

Throughout the study, 2,3-DPG concentrations were higher in the control group than in the "treated" group, but differences in the mean values did not reach statistical significance. Mean arterial blood gas and extracellular and intracellular pH measurements obtained while the patients were breathing air, at rest, did not differ significantly between the control and "treated" patients during the study, and there was no difference in MCHC values.

Of the initial 13 patients who were given domiciliary oxygen therapy, seven survived for three years or longer. By allowing each of these patients to serve as his own control, the effect of oxygen therapy within this group can be seen. The mean values of measurements made in these seven patients before and one month, one year, two years and three years after beginning oxygen therapy are shown in Table 3 .

Mean $P50_{(7.4)}$ determined after one month of oxygen therapy, 28.0 $\pm$ 2.1 mm Hg) was significantly higher ($P < 0.02$) than the mean pre-treatment $P50_{(7.4)}$ (26.8 $\pm$ 1.2 mm Hg), but the mean $P50_{(7.4)}$ determined after one, two and three years did not differ significantly from the mean pre-treatment value in those seven patients. In addition, the shape of the oxygen dissociation curve, indicated by Hill's n, did not change significantly during the study.

No other variable showed significant change from the pre-treatment value after only one month of oxygen therapy. After 12 months of domiciliary oxygen therapy, however, 2,3-DPG concentration (13.6 $\pm$ 3.24 μmoles/gHb) was significantly lower ($P < 0.05$) than the initial mean value (15.91 $\pm$ 1.75 μmoles/gHb), and this decrease

continued, as shown by the mean concentration at the second year assessment (13.43 ± 2.21 μmoles/gHb). However, by the end of the study, mean 2,3-DPG concentration began to rise again, and at the thir year assessment was 14.78 ± 3.47 μmoles/gHb, which was not significantly different from the initial value (Fig.12).

Haemoglobin concentration after one year of oxygen therapy, 13.8 ± 2.0 g/dl),was significantly lower ($P < 0.05$) than the mean pre treatment level (15.7 ± 1.7 g/dl) and this reduction was maintained at the second ($P < 0.02$) and third year ($P < 0.01$) assessments (13.6 ± 1.4 g/dl and 13.1 ± 1.6 g/dl respectively) (Fig. 13). The mean packed cell volume was associated with the changes in haemoglobin concentration throughout the study, being significantly lower than the average pre-treatment value (53.5 ± 7.8) at the first year (45.1 ± 10.0, $P < 0.025$), second year (45.0 ± 4.8 , $P < 0.02$) and third year assessments (44.0 ± 5.3 , $P < 0.005$) (Fig. 14).

Because the changes in packed cell volume were associated with the changes in haemoglobin concentration, there was no significant change in the M.C.H.C. during the course of the study.

Arterial PO_2, measured while the patients were breathing air, was not significantly different from the initial value (54.3 ± 5.3 mm Hg) after one year of oxygen therapy, but thereafter the degree of hypoxaemia appeared to increase, as judged by arterial PO_2 measurements made at the second and third year assessments (46.6 ± .6 mm Hg, $P < 0.02$, and 46.0 ± 10.0 mm Hg, $P < 0.10$ respectively) Fig. 15). However, the mean arterial PCO_2 and pH measurements did not differ from the initial pre-treatment values during the study.

As only two patients from the control group were alive after three years of the study, statistical evaluation of changes

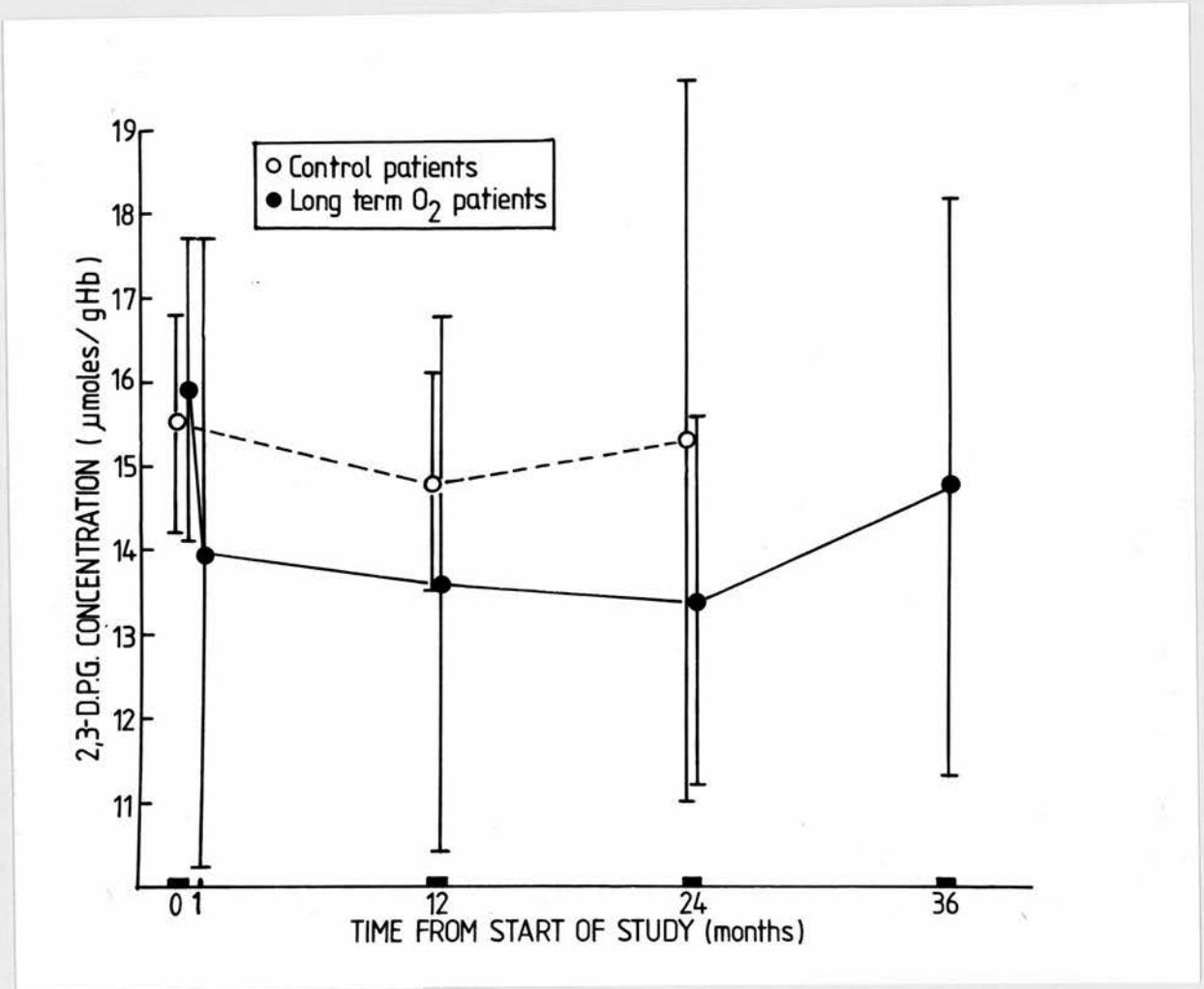


Figure 12

Mean 2,3-DPG concentrations (+ 1 S.D. in the 5 control group patients who survived for at least 2 years of the study (o), and in the 7 O_2-therapy patients who survived for 3 years of the study (●). Measurements in the control group were made initially, and after 1 and 2 years. Measurements in the O_2-therapy group were made initially and after 1 month, 1, 2 and 3 years.

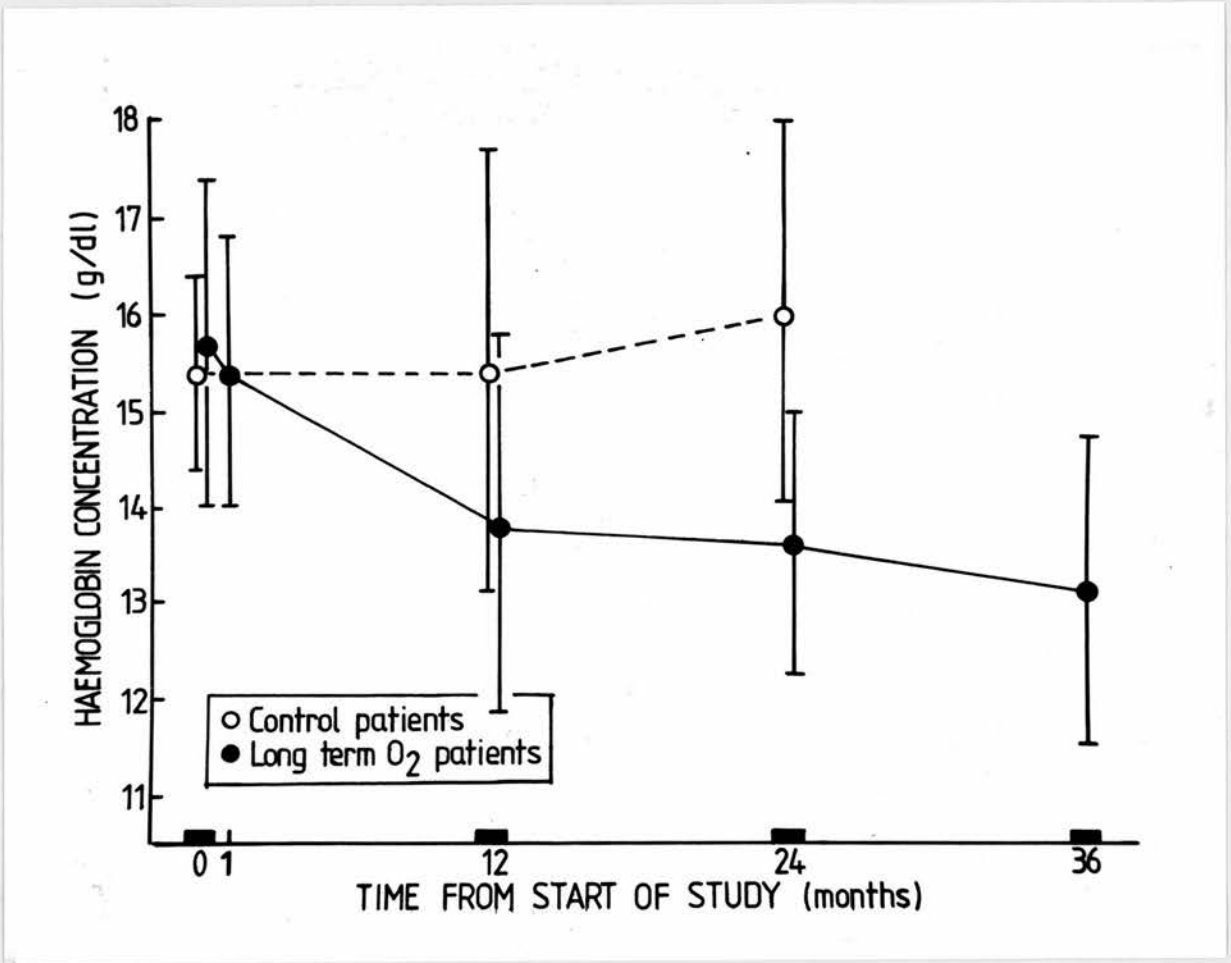


Figure 13

Mean haemoglobin concentrations (+ 1 S.D.) in the 5 control group patients who survived for at least 2 years of the study (o), and in the 7 O_2-therapy patients who survived for 3 years of the study (●). Measurements were made initially and after 1 and 2 years in the control group, and initially and after 1 month, 1, 2 and 3 years in the O_2-therapy group.

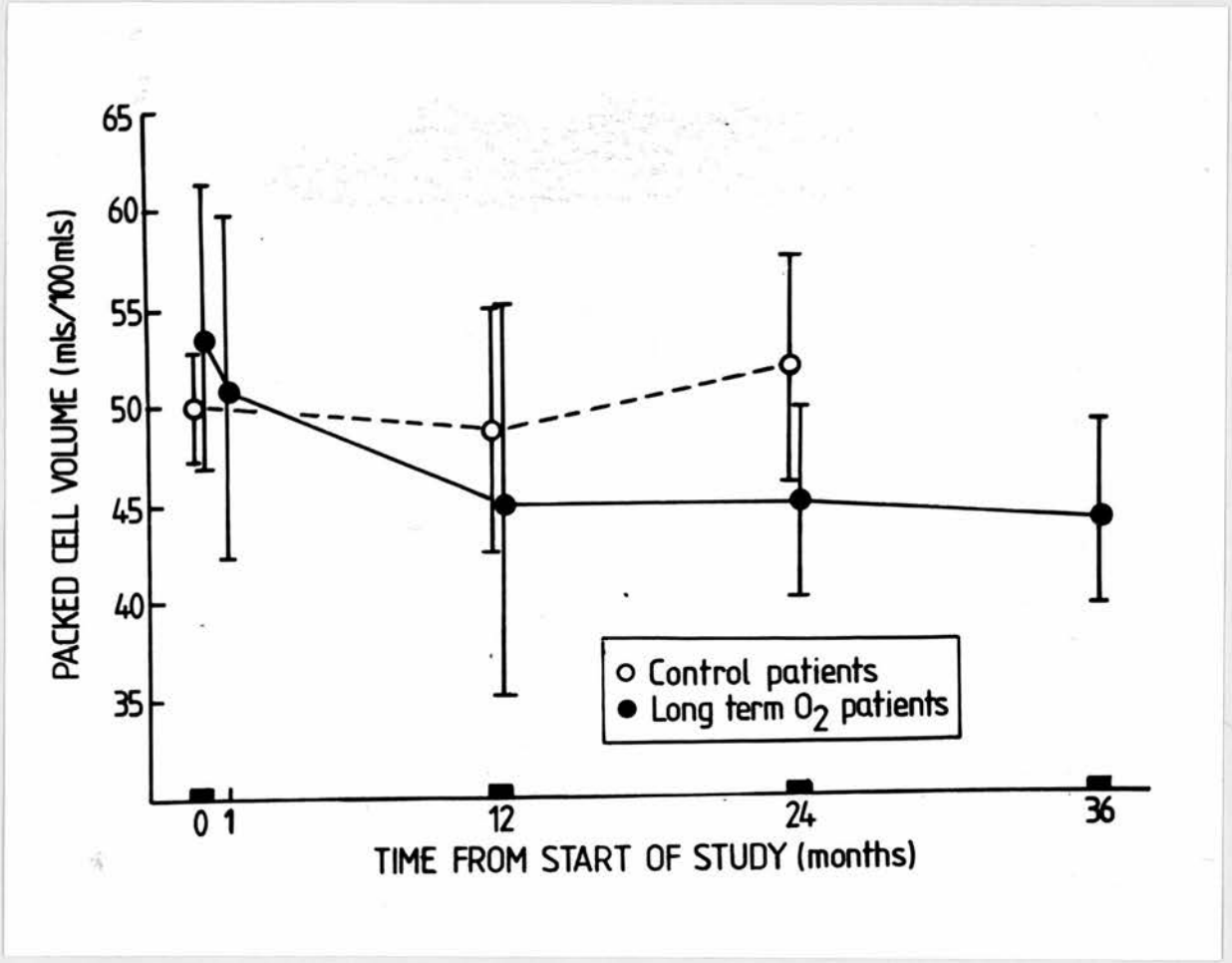


Figure 14

Mean P.C.V. (+ 1 S.D.) in the 5 control group patients who survived for at least 2 years of the study (o), and in the 7 O_2-therapy patients who survived for 3 years of the study (●). Measurements were made initially and after 1 and 2 years in the control group, and initially and after 1 month, 1, 2 and 3 years in the O_2-therapy group.

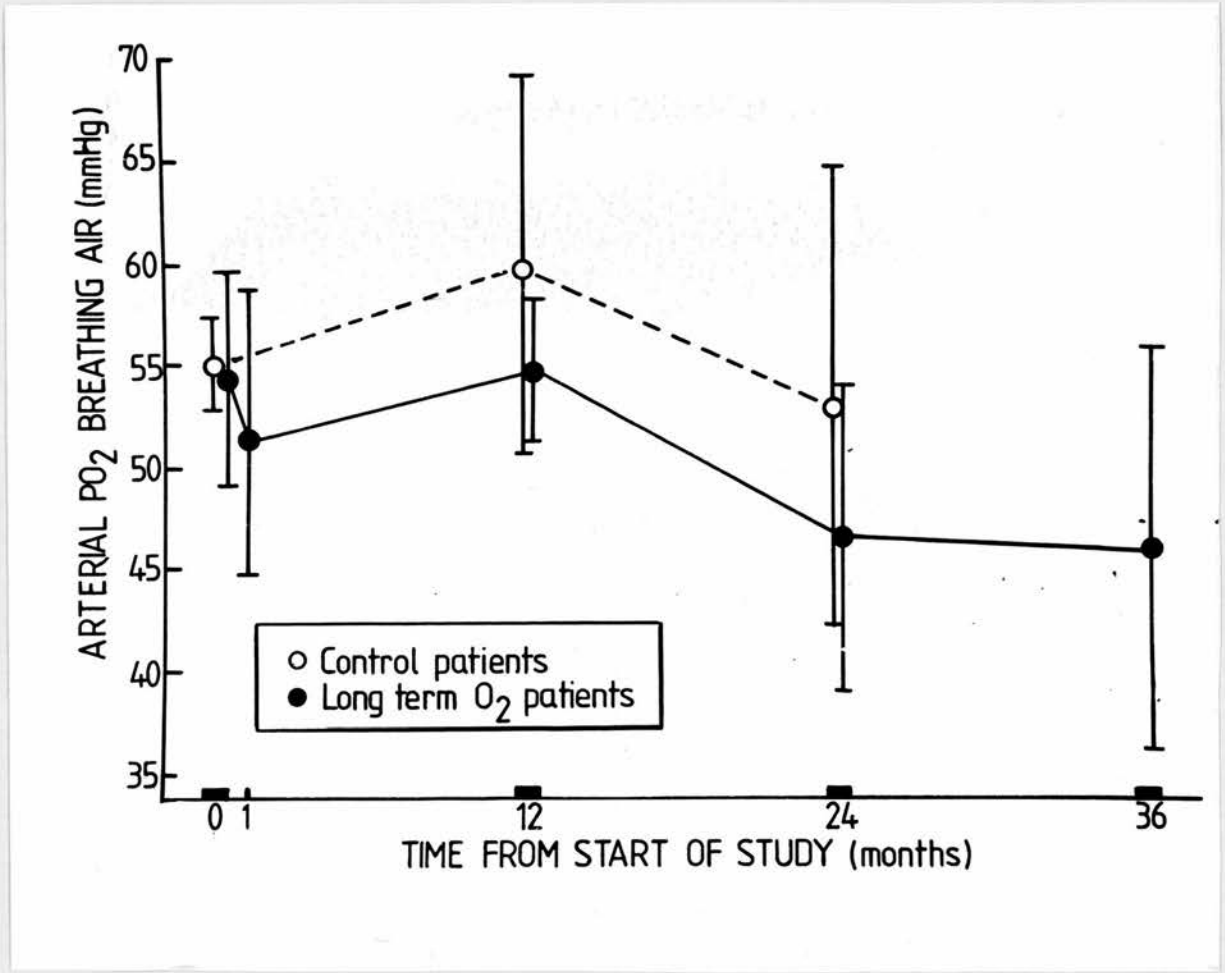


Figure 15

Mean arterial PO_2 (± 1 S.D.) in the 5 control group patients who survived for at least 2 years of the study (o), and in the 7 O_2-therapy patients who survived for 3 years of the study (●). Measurements were made initially and after 1 and 2 years in the control group, and initially and after 1 month, 1, 2 and 3 years in the O_2-therapy group.

occurring in the control group during the study was carried out on results from the five patients surviving at the second year assessment. During the two years, the only variable to change significantly in these patients was $P50_{(7.4)}$, which fell significantly ($P < 0.02$), from the mean pre-treatment value (27.0 ± 1.9 mm Hg) after the first year (26.5 ± 0.5 mm Hg), but increased again to the pre-treatment value after two years (27.2 ± 1.5 mm Hg).

All other variables in the control group did not change significantly during the study, mean values of 2,3-DPG, haemoglobin, packed cell volume and arterial PO_2 during the study being shown in Figs. 12, 13, 14 and 15 and Table 3.

From serial measurements of carboxyhaemoglobin levels in those patients receiving oxygen therapy, it was apparent that six of the eleven patients alive after one year of the study continued to smoke, as their carboxyhaemoglobin levels were repeatedly greater than 4%. Allowing each patient to serve as his own control, differences in response to 12 months of oxygen therapy, between the smokers and non-smokers, can be seen. In the five non-smoking patients, the mean haemoglobin concentration after one year of oxygen therapy (13.2 ± 1.45 g/dl) was significantly lower ($P < 0.01$) than the pre-treatment mean in those patients (15.8 ± 1.69 g/dl). However, although the mean haemoglobin concentration in the six patients who continued to smoke was lower after one year of oxygen therapy (14.9 ± 2.11 g/dl) than it was at the start of the study (16.21 ± 2.49 g/dl), the fall in haemoglobin concentration was less dramatic ($P < 0.10$) (Fig. 16). Similarly, the fall in the mean packed cell volume in the

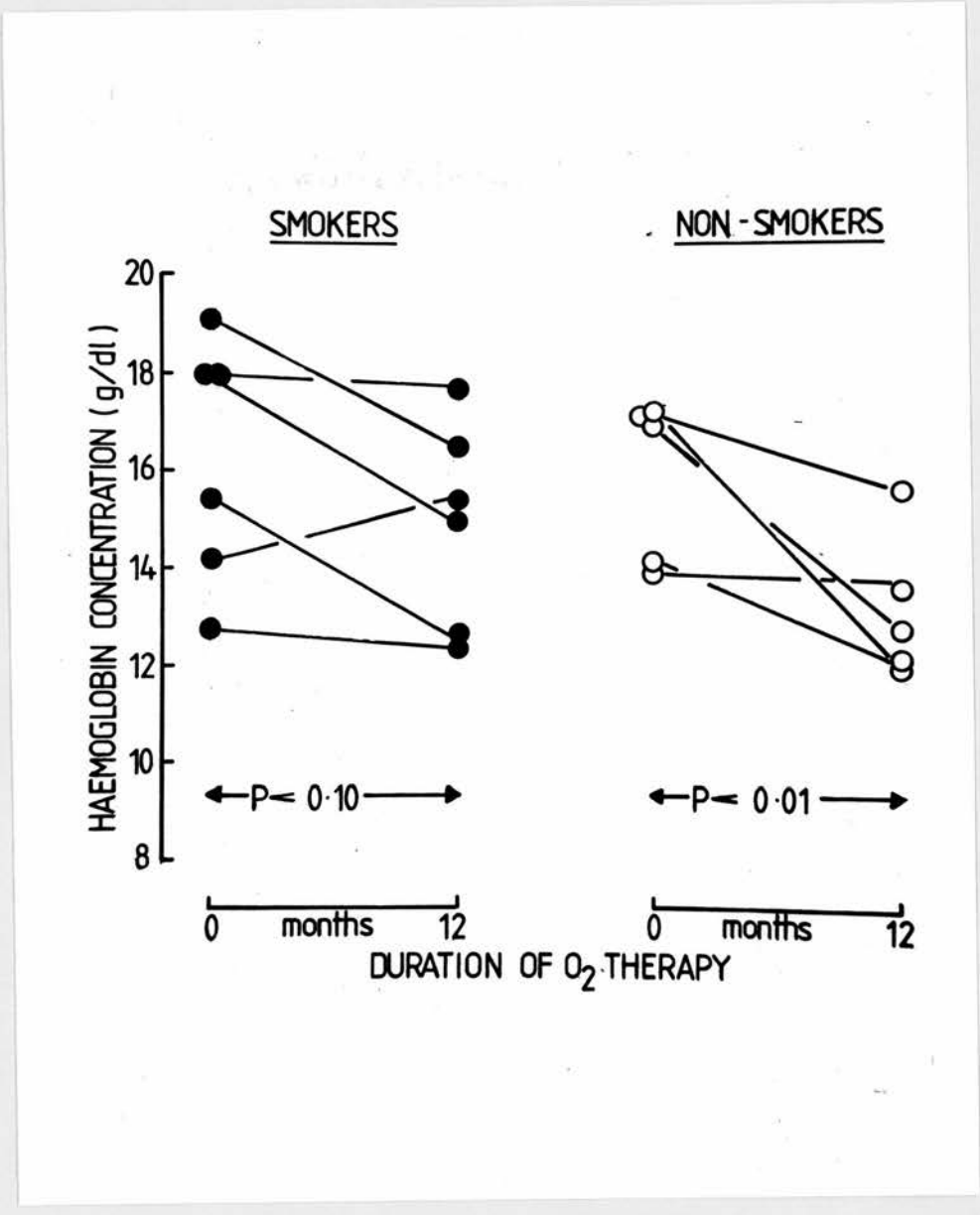


Figure 16

Haemoglobin concentration determined before, and 12 months after beginning long term O_2 therapy in 5 non-smoking (o), and 6 smoking patients (•) with chronic bronchitis.

non-smoking patients (from 53.6 ± 6.68 initially, to 42.2 ± 4.20 after one year) was highly significant ($P < 0.001$), but the fall in mean packed cell volume in the smoking patients (from 54.7 ± 9.35 initially, to 49.9 ± 10.5 after one year) only reached statistical significance at the 10% level (Fig. 17).

The concentration of 2,3-DPG did not change significantly after one year of oxygen therapy in those patients who continued to smoke (mean pre-treatment value 15.21 ± 1.61 μmoles/gHb, mean value after one year 15.18 ± 2.28 μmoles/gHb), but in the non-smoking patients, mean 2,3-DPG concentration fell markedly ($P < 0.10$) from the pre-treatment value (15.85 ± 1.90 μmoles/gHb) after one year of oxygen therapy (13.52 ± 1.60 μmoles/gHb) (Fig. 18).

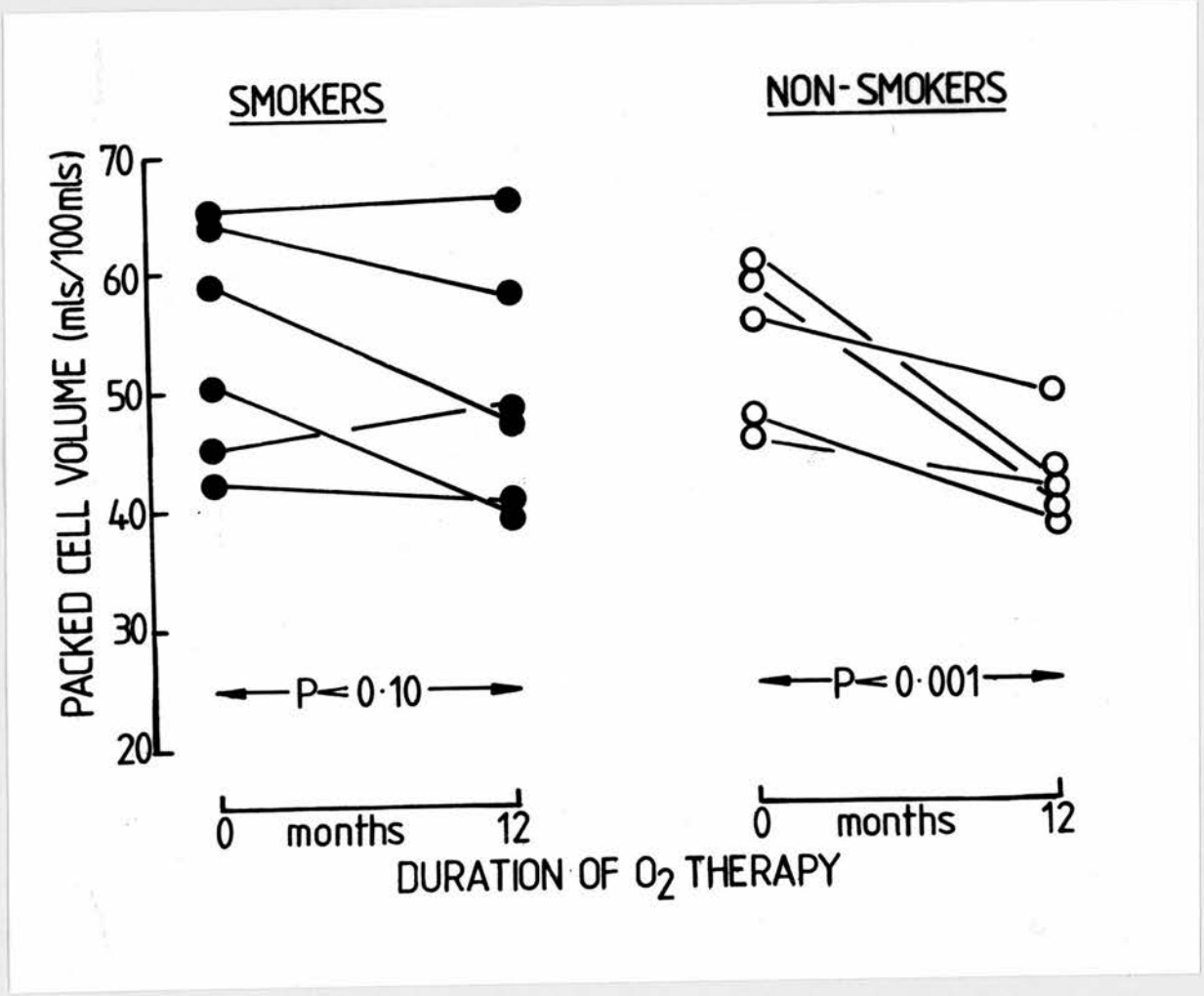


Figure 17

Packed cell volume determined before and 12 months after beginning long term 0_2 therapy in 5 non-smoking (o), and 6 smoking patients (•) with chronic bronchitis.

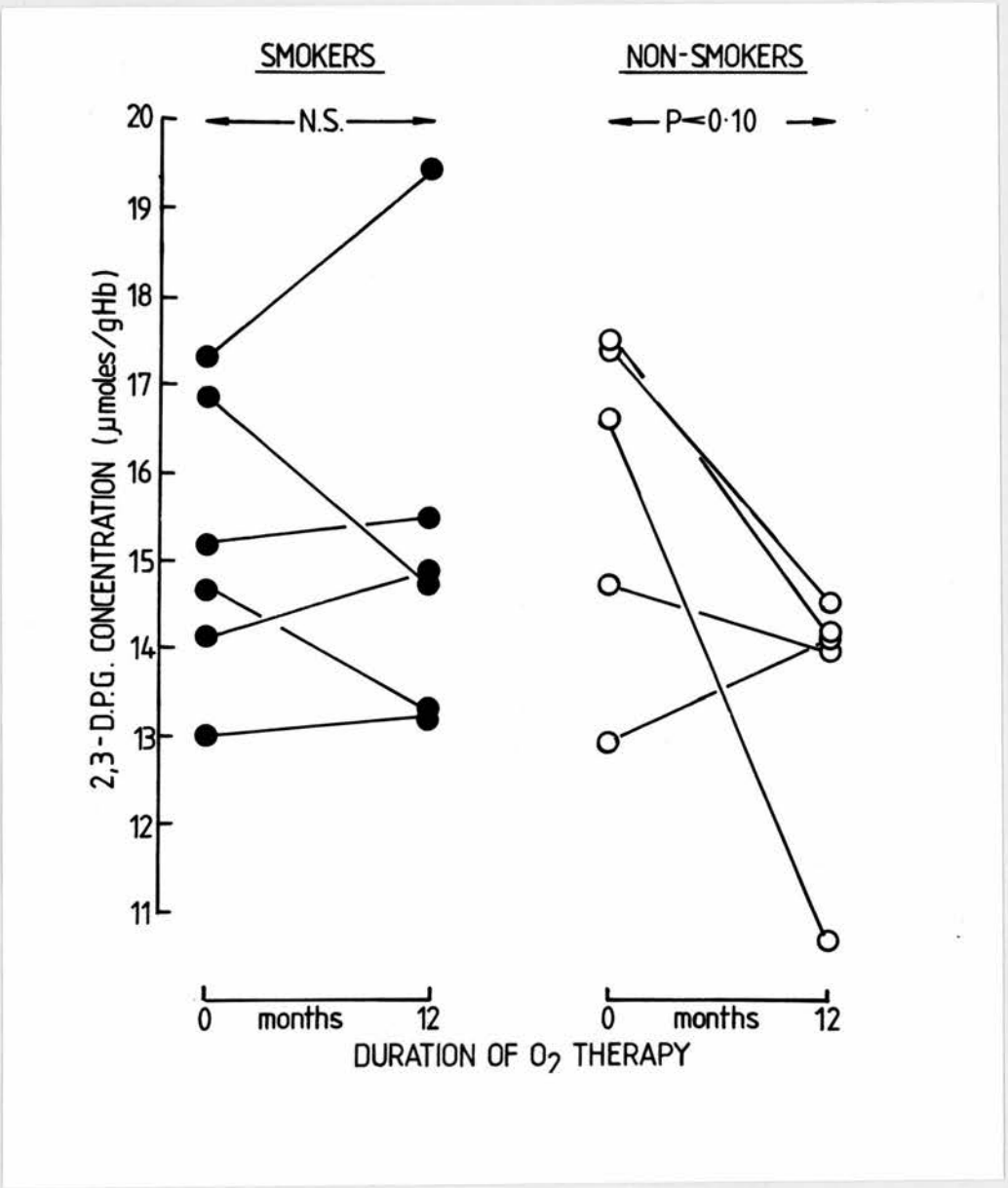


Figure 18

2,3-DPG concentration determined before, and 12 months after beginning long term 0_2 therapy in 5 non-smoking (o) and 6 smoking patients (•) with chronic bronchitis.

Discussion of results appertaining to the study of long term oxygen therapy

The results of this controlled study of long term oxygen therapy confirm and extend observations made in previous uncontrolled studies of oxygen therapy in patients with chronic obstructive lung disease. Viewing each patient who received oxygen therapy as his own control, significant reductions in haemoglobin concentration and packed cell volume noted by previous investigators (Chamberlain and Millard, 1963; Block, Castle and Keitt, 1974) were confirmed in this study, but the beneficial effect of oxygen becomes more convincing when haemoglobin concentration and packed cell volume measurements are compared with those in the matched control patients who showed no reversal of the compensatory polycythaemic response. This reduction of haemoglobin concentration in patients receiving oxygen therapy would necessarily reduce the oxygen capacity of the blood, but this decrease is offset in arterial blood by the increased oxygen saturation afforded by oxygen therapy. Thus, as the mean haemoglobin concentration falls from 15.7 g/dl to 13.1 g/dl, as in patients in this study, the oxygen capacity is reduced from 21.0 mls/100 ml blood to 17.6 mls/100 ml blood (assuming an oxygen combining power of 1.34 mls/gHb), but if the mean oxygen saturation is raised from 80 - 92% by oxygen therapy, the arterial oxygen content will only decrease by 0.6 mls/100 ml blood, from 16.8 to 16.2 mls/100 ml blood. Similarly, when each patient receiving oxygen therapy serves as his own control, serial measurements of 2,3-DPG reveal the reversal of another compensatory phenomenon. A number of authors have suggested that 2,3-DPG concentration increases in response to the hypoxic stimulus (Lenfant, Ways/Aucutt and Cruz, 1969; Oski,

Gottlieb, Miller and Delivoria-Papadopoulos, 1970; Oski, Gottlieb, Delivoria-Papadopoulos and Miller, 1969), but this has not been supported by other studies (Edwards and Canon, 1972; Guy, Kazura, Bromberg and Balcerzak, 1971). The mean 2,3-DPG concentration in patients in this study was at the upper limit of the normal range in our laboratory (12.44 - 16.28 μmoles/g haemoglobin) at the beginning of the study. However, after one year of oxygen therapy the 2,3-DPG concentration in the patients receiving oxygen treatment had fallen significantly to within the normal range, and it remained at this level for two years. This observation confirms that of Block et al (1974) who found a slight decrease in 2,3-DPG concentration in patients with chronic airways obstruction during a four week period of oxygen therapy, although these changes did not reach statistical significance.

The fall in 2,3-DPG concentration was not reflected by an increase in the oxygen affinity of the blood, assessed by $P50_{(7.4)}$ measurements. However, using data from Arturson, Garby, Robert and Zaar (1974), the theoretical effect of this decrease in 2,3-DPG concentration on $P50_{(7.4)}$ could be calculated. These authors determined ligand correction factors for $\Delta \log PO_2$ (mm Hg)/ Δlog DPG (mmole/l red cells) for constant oxygen concentration, pH and PCO_2. Their calculations, therefore, neglect the effect of changing 2,3-DPG concentrations on red cell pH, but as our results show little change in the red cell pH during the study, these correction factors serve as a close approximation. For an oxygen saturation of 50%, pH 7.40 and PCO_2 40 mm Hg, the ligand correction factor, $\Delta \log PO_2 / \Delta \log$ DPG, is 0.31. If this is then applied to the change in mean 2,3-DPG concentration observed after one year of oxygen therapy, the change in PO_2 at 50%

oxygen saturation and pH 7.4 (i.e. $P50_{(7.4)}$) would be only 0.9 mm Hg. When variations normally present in blood gas, pH and oxygen saturation measurements are considered, it is not too surprising that no fall in $P50_{(7.4)}$ was detected.

The determination of in vitro $P50_{(7.4)}$ involves the use of correction factors to standardise the measurement to a pH 7.40, PCO_2 40 mm Hg and zero base excess. The formula of Severinghaus (1966) was employed for these corrections, and is approximated by: $\Delta \log PO_2 = -0.48 \ \Delta$ pH + 0.0013 Base Excess. This formula corrects for deviations in pH from 7.40 produced by respiratory and metabolic causes. Inherent in the use of this correction factor is the assumption that all individuals respond in an identical way to changes in pH and base excess. This assumption will necessarily introduce errors into the calculations. In addition, the use of Severinghaus's correction formula assumes that the extracellular pH mimics pH changes within the red cell, for it is the latter which influences oxygen affinity. By simultaneous measurements of extra- and intracellular pH in this and other studies (Fairweather, Walker and Flenley, 1974; Tweeddale, Leggett and Flenley, 1977), this assumption appears justified.

From measurements of $P50_{(7.4)}$ and Hill's 'n', it appears that long term oxygen therapy has no significant effect on the position or shape of the oxygen dissociation curve in patients with chronic airways obstruction. In both the control group patients and those patients receiving oxygen therapy, the mean $P50_{(7.4)}$ determined before and during the study remained within the normal range for subjects of the same age group (Tweeddale, Leggett and Flenley, 1976). This is contrary to the findings of

Block et al (1974), who investigated the rate of oxygen release from haemoglobin and found it to be increased in patients with chronic airways obstruction when breathing air, but returned to normal after four weeks of domiciliary oxygen therapy. The findings of Block et al, in part, support those of Lenfant et al (1969), who found raised $P50_{(7.4)}$ values in patients with chronic airways obstruction, complicated by polycythaemia, but Fairweather et al (1974) and Tweeddale et al (1977) were unable to demonstrate any difference in $P50_{(7.4)}$ between normal subjects and patients with obstructive lung disease.

Similarly, the slope of the oxygen dissociation curve, expressed as Hill's 'n' was unaffected by oxygen therapy, and remained normal in both groups of patients during the study, suggesting that haem-haem interaction is not altered by hypoxaemia.

Serial measurements of arterial blood gases indicate that long term oxygen therapy does not improve the underlying lung condition in patients with chronic bronchitis, as arterial PO_2, measured while breathing air, did not improve in those patients receiving oxygen treatment, nor did it differ significantly from measurements made in the control group patients during the study. However, further results from the whole M.R.C. trial of long term oxygen therapy (In Press, October 1980) show that long term oxygen administration appeared to prevent a further fall in the arterial PO_2 when the patients were breathing air. Measurements of arterial PCO_2 indicate that oxygen, administered at a flow rate of 2 litres/minute for 15 hours in the day did not cause chronic O_2 retention.

The results of this study indicate that domiciliary oxygen therapy can reverse a number of compensatory responses caused by

hypoxaemia. However, a comparison of the effectiveness of 12 months of oxygen therapy in patients who continued to smoke and those who discontinued this habit on commencement of oxygen therapy, shows an incomplete response to oxygen therapy in the smokers, and confirms the findings of Foster, Corrigan and Goldman (1978). Many authors have demonstrated above average levels of red cell mass, haemoglobin concentration and haematocrit resulting from impaired tissue oxygen supply in otherwise normal healthy subjects who smoked (Sagone, Lawrence and Balcerzak, 1973; Sagone and Balcerzak, 1975; Smith and Landaw, 1978). On the basis of these results, it is likely that oxygen therapy would be less efficient in improving tissue oxygen supply, and removing the erythropoietic response in patients with chronic bronchitis and secondary polycythaemia, if the patient continued to smoke, and thereby maintained the low oxygen combining power of the blood, and a comparison of the effectiveness of oxygen therapy in the non-smoking and smoking patients substantiates this hypothesis by showing an incomplete reversal of elevated levels of haemoglobin concentration and packed cell volume after 12 months oxygen therapy in the patients who continued to smoke.

Sagone et al (1973 and 1975) and Smith and Landaw (1978) found no compensatory rise in 2,3-DPG concentration in subjects with carbon monoxide-induced hypoxaemia, but our results show that the fall in 2,3-DPG concentration, observed in the non-smoking patients after 12 months of oxygen therapy, did not occur in those patients who continued to smoke. Existing theories of 2,3-DPG regulation cannot explain this lack of response to oxygen therapy amongst the smoking patients, for there was no significant difference in the pH of the blood between the smokers and non-smokers which may have altered the activity of enzymes in the Rapoport-Leubering shunt, or was there a greater amount of reduced haemoglobin in the smokers,

producing an increase in potential 2,3-DPG binding sites, thereby allowing increased synthesis of the free compound.

Similarly, Dinman, Eaton and Brewer (1970) who found 2,3-DPG levels in humans rose after acute exposure to carbon monoxide, but remained stable in control subjects, were unable to explain their results using existing theories of 2,3-DPG regulation, and suggested the existence of a hypoxia-driven stimulation of 2,3-DPG synthesis, independent of the deoxy-haemoglobin-binding mechanism, and the results of our investigation appear to support this suggestion.

CHAPTER 5

Study of the Role of Carboxyhaemoglobin in the Genesis of Secondary Polycythaemia in Chronic Bronchitis

Subjects and experimental design

Forty-seven patients (20 women, 27 men) with chronic hypoxic cor pulmonale were studied. They were all hypoxaemic (arterial PO_2 43 - 62 mm Hg), and all, except six, were hypercapnic (arterial PCO_2 33 - 68 mm Hg) when breathing air at rest, and all had severe airways obstruction ($FEV_{1.0}$ 0.6 $\pm$ 0.2 L). The patients were in a stable clinical state when studied, as judged by constant weight, $FEV_{1.0}$ and arterial blood gas tensions over a four week period prior to the study.

Seventeen patients were classified as non-smokers on the basis of their smoking history, together with measurements of their carboxyhaemoglobin levels. A carboxyhaemoglobin saturation below 4% was accepted as evidence that the patient was no longer smoking, and patients were only classified as non-smokers if they had not smoked for two years prior to the study.

The degree of secondary polycythaemia was assessed by red cell mass and plasma volume determinations using IV autologous ^{51}Cr labelled red blood cells and ^{131}I labelled albumin (Hurley, 1975), and oxygen transport was assessed by measurements of the P50 and haemoglobin concentration, together with the arterial blood gas tensions and pH. From this data the arterial oxygen saturation was determined in two ways: firstly, by assuming a normal oxygen dissociation curve in the absence of any carboxyhaemoglobin, the oxygen saturation could be obtained directly from the Severinghaus standard oxygen dissociation curve ($P50_{(7.4)}$ = 26.6 mm Hg), corrected for arterial pH; secondly, the oxygen saturation was determined using the oxygen dissociation curve, obtained for

each patient. This curve, which was usually constructed during the period of hospital admission when the carboxy-haemoglobin levels in all patients were low, was corrected to take into account the effect of the average carboxy-haemoglobin levels present in the patients at the frequent outpatient visits (Collier, 1976), and the oxygen saturation corresponding to the arterial oxygen tension and pH was determined. This saturation can be expressed in two ways: as a percentage of total haemoglobin (SO_2T), or as a percentage of available haemoglobin (SO_2A), the latter expression excluding that haemoglobin in combination with carbon monoxide. In non-smokers where carboxyhaemoglobin levels are low, there is little difference, numerically, between SO_2T and SO_2A. However, as carboxyhaemoglobin levels increase, the difference widens. Using oxygen saturation, expressed as a percentage of total haemoglobin concentration, arterial oxygen content (CaO_2) was estimated:-

$$CaO_2 = \frac{SO2T}{100} \times O_2 \text{ capacity}$$

where O_2 capacity = Hb (g/dl) x 1.34

The probable level of oxygen content in the renal venous blood (CvO_2) was then calculated on the assumption that renal arterio-venous oxygen content difference was either 2 or 5 ml O_2/100 ml blood. This renal venous oxygen content was then expressed as saturation (SvO_2):-

$$SvO_2 = \frac{Cv02}{O_2 \text{capacity}} \times 100$$

This saturation value could then be used to derive the renal venous oxygen tension (PVO_2) using the corrected oxygen dissociation curve.

Significance of differences of mean values between the smokers and non-smokers were determined by the unpaired 't' test.

Results appertaining to the study of polycythaemia in chronic hypoxic cor pulmonale

There was no significant difference in age, resting arterial blood gas tensions or pH between those patients who smoked and those who were non-smokers (Table 4). Similarly, there was no significant difference between mean arterial oxygen saturation measurements in the two groups, when expressed as a percentage of the available haemoglobin (SO_2A). However, arterial oxygen saturation expressed as a percentage of the total haemoglobin (SO_2T, which includes the COHb concentration) was significantly higher ($P < 0.02$) in the non-smoking patients (mean 83.9 $\pm$ 4.2%) than in the smokers (mean 80.1 $\pm$ 5.4%) and mean red cell mass measurements were significantly lower ($P < 0.001$) in the non-smokers (mean 29.2 $\pm$ 4.1 mls/kg) than in the smokers (mean 42.4 $\pm$ 8.0 mls/kg) (Table 5).

In those patients who smoked, there was a significant linear relationship between red cell mass and arterial oxygen saturation when determined assuming a normal oxygen dissociation curve with no COHb present ($r = -0.38$, $P<0.05$), but as shown in Fig. 19 there was wide variability in the polycythaemic response to oxygen saturation determined in this way. This relationship improved only slightly when SO_2A, determined after correction of the measured oxygen dissociation curve for the prevailing mean COHb level in each patient was used ($r = -0.40$, $P < 0.05$). However, when SO_2A was replaced by SO_2T, which reflects the lower combining ability of haemoglobin and oxygen in the presence of COHb, the correlation improved markedly ($r = -0.56$, $P < 0.005$), indicating a strong inverse relationship between red cell mass and SO_2T (Fig. 20). In the non-smoking patients, no significant relationship

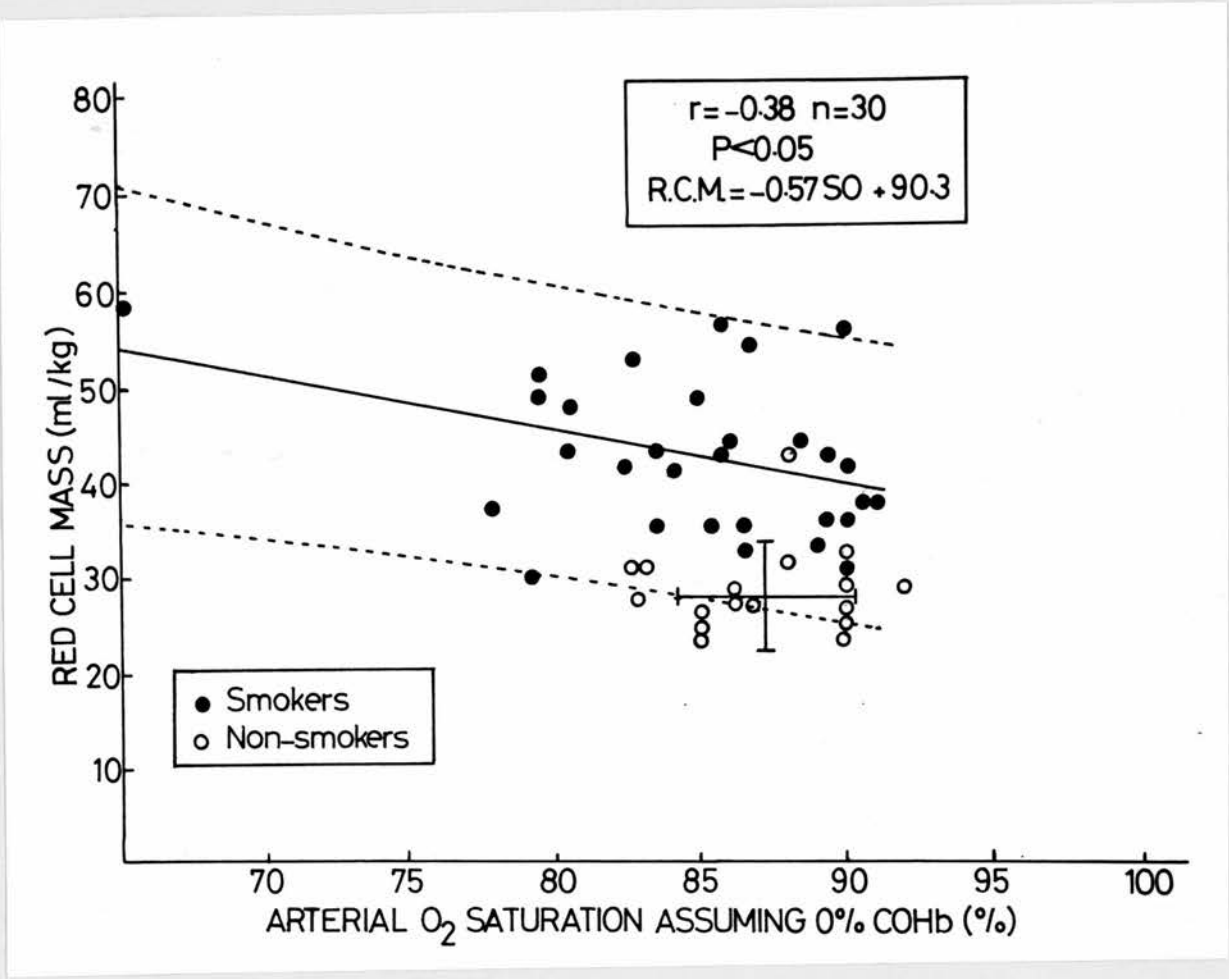


Figure 19

Red cell mass is plotted against arterial oxygen saturation calculated assuming a normal oxygen dissociation curve with 0% COHb, for non-smoking (o) and smoking patients (●) with chronic bronchitis. The regression line relates to the smokers ($r = -0.38$, $P < 0.05$), and the mean value $\pm$ 1 S.D. to the non-smokers

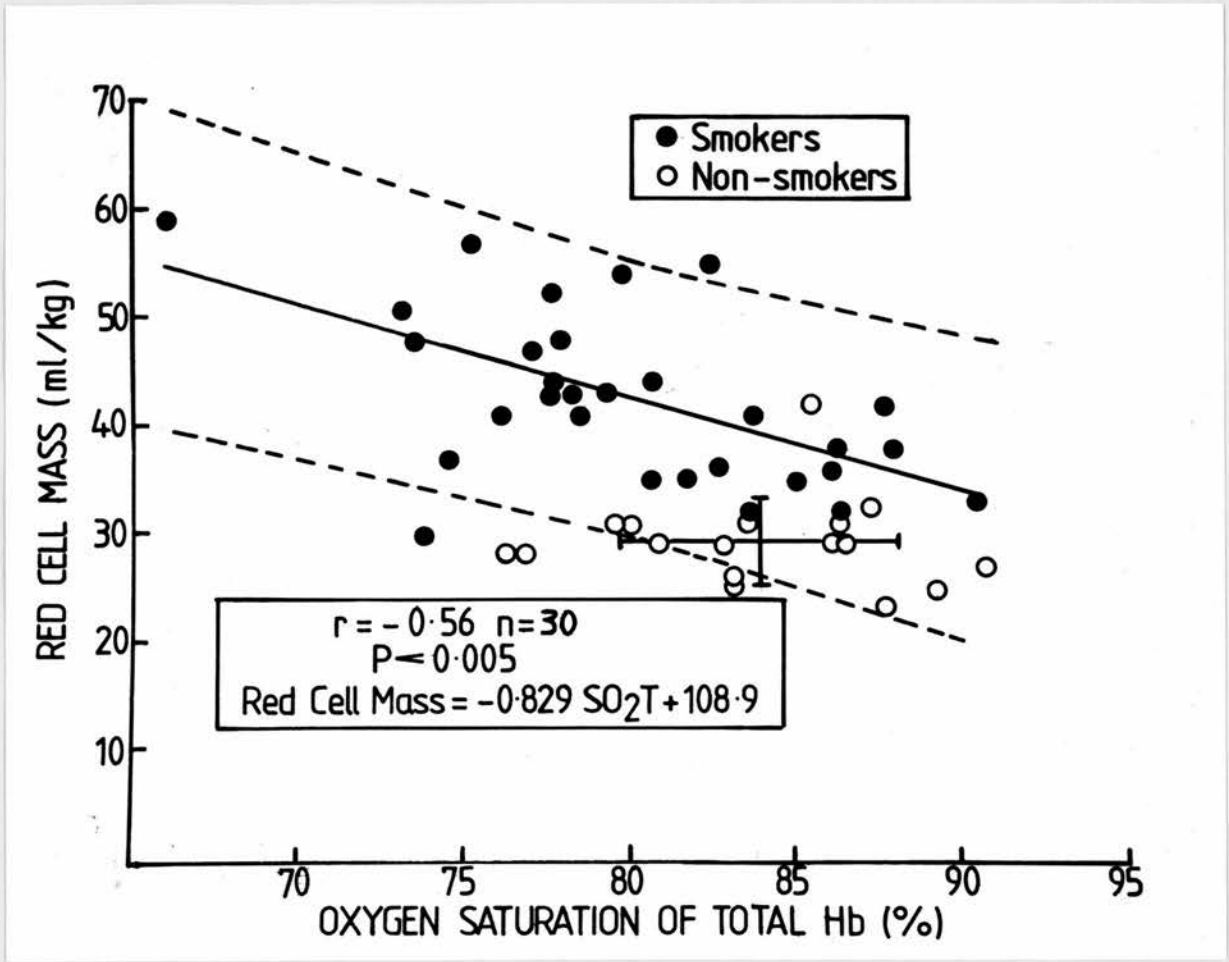


Figure **20**

Red cell mass is plotted against arterial oxygen saturation, determined taking the mean COHb level in each patient into account, and expressed as a percentage of the total haemoglobin concentration (SO_2T) for the non-smoking patients (o), and the smoking patients (•) with chronic bronchitis. The regression line relates to the smokers ($r = -0.56$, $P < 0.005$), and the mean value $\pm$ 1 S.D. to the non-smokers.

was found between red cell mass and oxygen saturation when determined assuming COHb levels of 0% ($r = 0.01$, $P > 0.10$) (Fig. **19**). Nor did a relationship between red cell mass and oxygen saturation emerge in the non-smokers when COHb levels were taken into account and oxygen saturation was expressed as SO_2A ($r = 0.02$, $P > 0.10$) or SO_2T ($r = 0.08$, $P > 0.10$) (Fig. **20**). The data presented in Figs. **19** and **20** not only indicates clearly different relationships between red cell mass and arterial oxygen saturation in the smokers and non-smokers, but also that the smokers tended to have a greater degree of polycythaemia for any given level of arterial oxygen saturation than did the non-smokers.

When red cell mass was related to the mean prevailing CoHb level in each patient, smoking and non-smoking, a significant linear correlation emerged ($r = 0.64$, $P < 0.001$). (Fig. **21**)

There was no significant difference in renal venous oxygen tensions, calculated using the measured $P50_{(7.4)}$ and assuming an arterio-venous oxygen content difference of 2 mls/100 ml blood, between those patients who smoked (mean $Pv0_2 = 39.3 \pm 4.0$ mm Hg) and the non-smoking patients (mean $Pv0_2 = 43.0 \pm 3.8$). However, assuming an arterio-venous oxygen content difference of 5 mls/100 ml blood, the renal venous oxygen tension in the non-smokers (mean value 33.2 ± 3.2 mm Hg) was significantly higher ($P < 0.05$) than that in the smokers (mean value 30.2 ± 3.0 mm Hg) (Table **5**).

Using data from all patients a significant negative linear relationship emerged between red cell mass and renal venous oxygen tension, calculated assuming an arterio-venous oxygen content difference of 2 mls/100 ml ($r = -0.48$, $P < 0.005$) (Fig. **22**), but

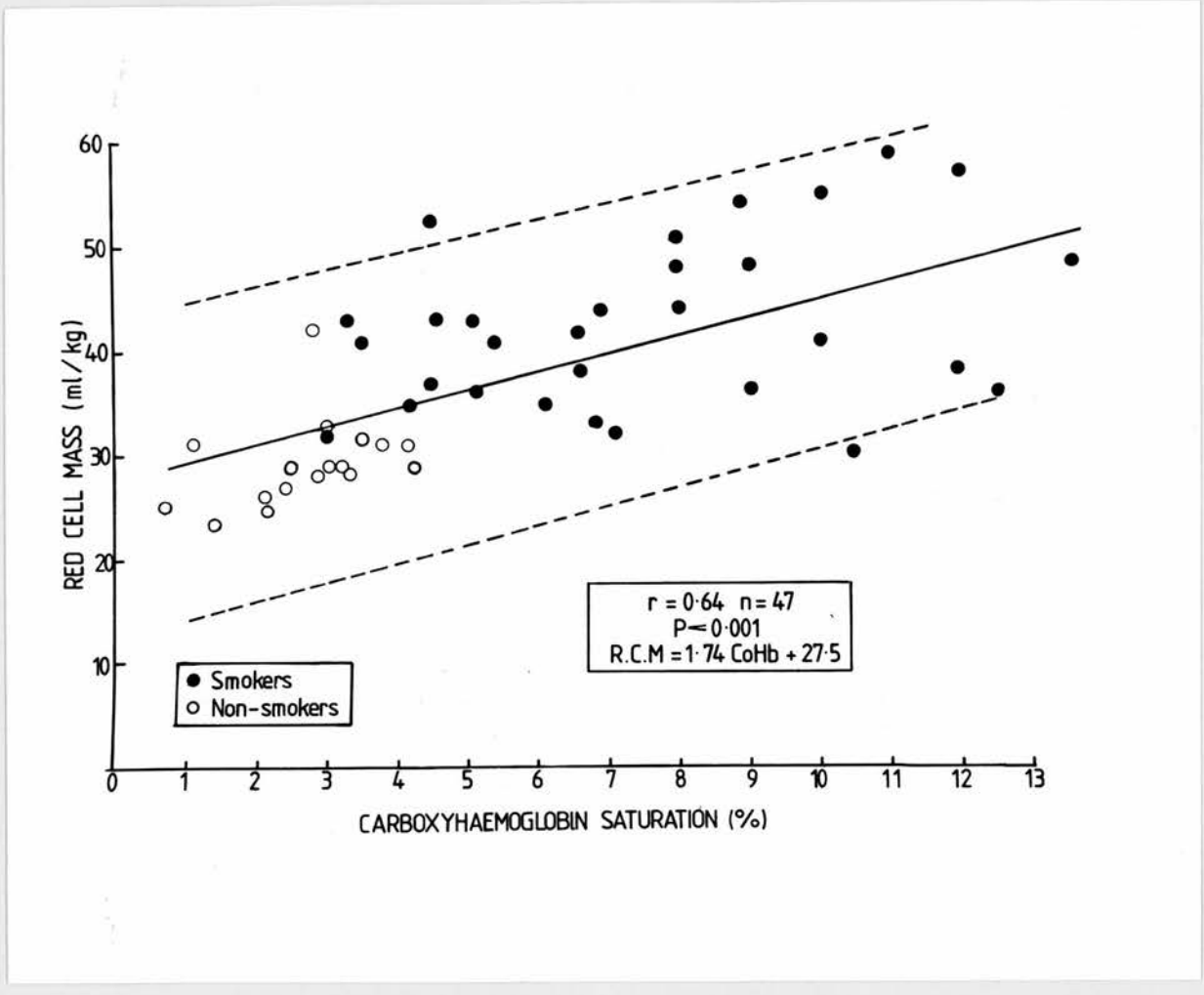


Figure 21

Red cell mass is correlated with COHb saturation in the non-smoking patients (o), and smoking patients (●) with chronic bronchitis ($r = 0.64$, $P < 0.001$).

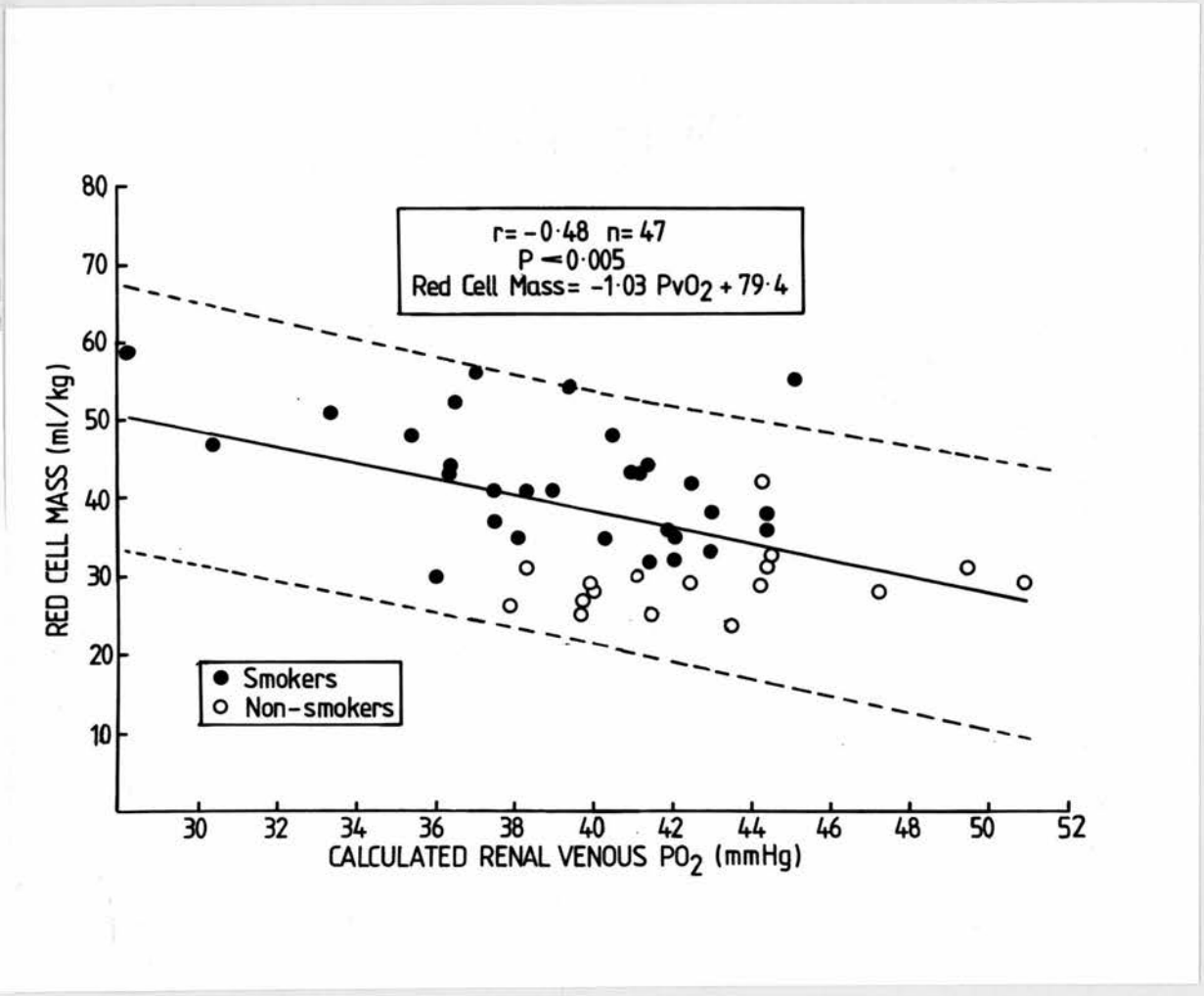


Figure **22**

Red cell mass is plotted against renal venous oxygen tension, calculated assuming an arterio-venous 0_2 content difference of 2 ml 0_2/100 ml blood in non-smoking (o), and smoking patients (●) with chronic bronchitis ($r = -0.48$, $P < 0.005$).

the relationship improved when red cell mass was related to the renal venous oxygen tension calculated assuming an arterio-venous oxygen content difference of 5 ml/100 ml ($r = -0.51$, $P < 0.001$) (Fig. **23**).

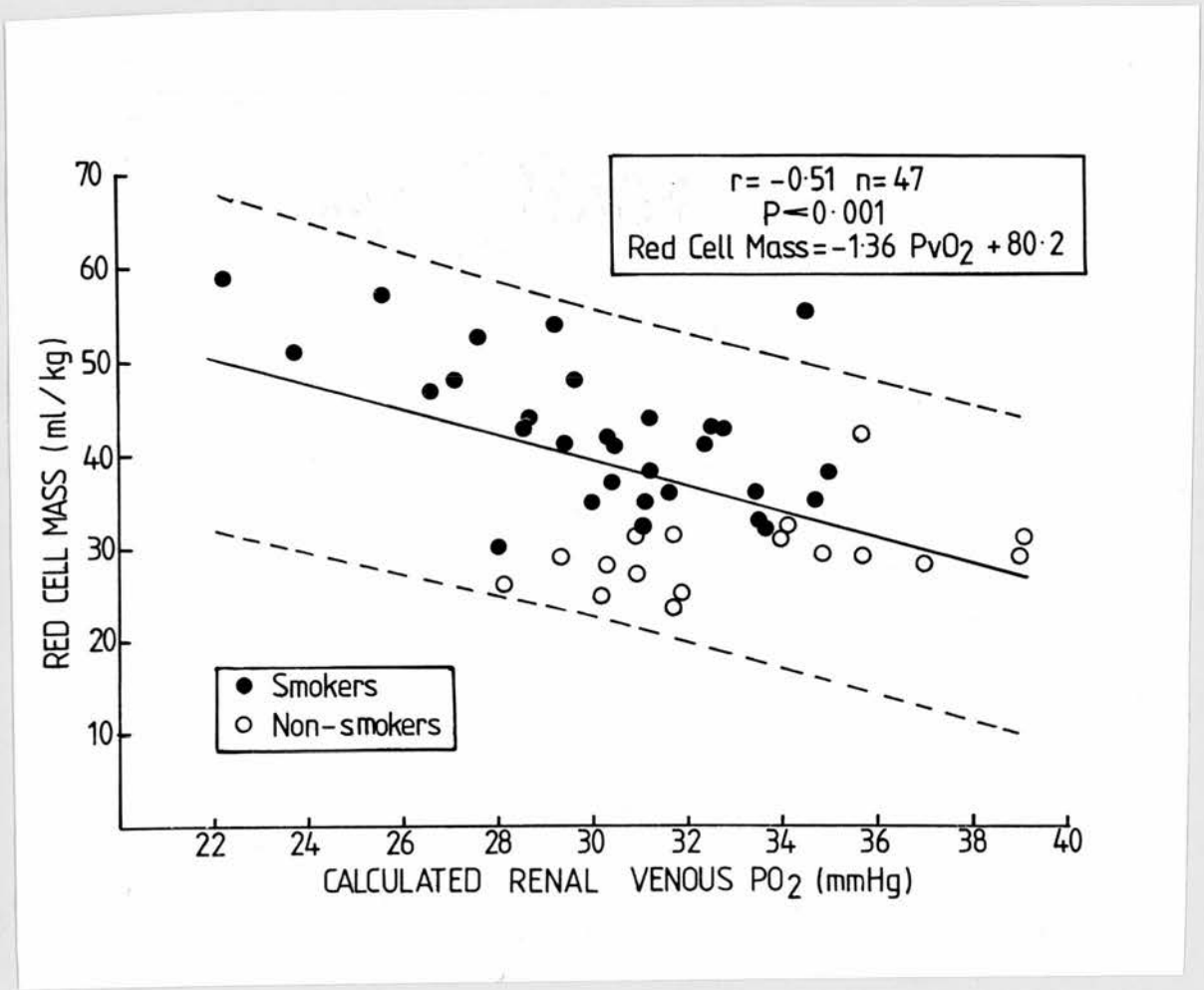


Figure 23

Red cell mass is plotted against renal venous oxygen tension, calculated assuming an arterio-venous O_2 content difference of 5 ml O_2/100 ml blood in non-smoking (o) and smoking patients (●) with chronic bronchitis ($r = -0.51$, $P < 0.001$).

Discussion of results appertaining to the study of polycythaemia in chronic hypoxic cor pulmonale

Previous studies carried out by Weil, Jamieson, Brown and Grover (1968), Chan(1969), and Harrison (1973), established a relationship between polycythaemia and hypoxaemia both in subjects at altitude and in patients with chronic bronchitis. However, the authors failed to take into account the smoking habits of the subjects when determining the arterial oxygen saturation, and assumed each subject had a normal oxygen dissociation curve and low carboxy-haemoglobin level. In our experience, patients who smoke may have carboxyhaemoglobin levels as high as 15%, and it is therefore possible that by omitting to take this into account, gross inaccuracies in the determination of oxygen saturation may have resulted, leading to wide variations in the response to hypoxaemia.

The results obtained in this study confirm the negative relationship between red cell mass and arterial oxygen saturation, as determined by the authors mentioned above, assuming no carboxy-haemoglobin, but our results showed this relationship was very variable (Fig. **19**). However, by taking into account the carboxyhaemoglobin levels normally present in these patients, much of the variation was removed, and the relationship between red cell mass and arterial oxygen saturation improved.

The data represented in Figure **20** indicates that for any given level of arterial oxygen saturation, values of red cell mass in the patients who smoked are greater than those found in the non-smoking patients. This observation suggests that the carboxyhaemoglobin formed by combination of haemoglobin with carbon monoxide present in cigarette smoke, enhances the

polycythaemic response to hypoxia by its dual effect on the haemoglobin molecule, that is by lowering the oxygen carrying capacity of haemoglobin, and by increasing the affinity of haemoglobin for oxygen, thus impairing tissue oxygen supply. This suggestion is strongly supported by the close correlation observed between red cell mass and carboxyhaemoglobin levels (Fig. **21**). Vanuxem, Guillot, Fornaris, Weiller and Grimaud (1977), and Foster, Corrigan and Goldman (1978) reached a similar conclusion from their work in patients with chronic respiratory insufficiency, but they assessed polycythaemia from haemoglobin concentration and haematocrit results, which may not reflect the extent of the polycythaemic response as accurately as measurements of red cell mass, as concomitant changes in plasma volume may conceal the extent of polycythaemia.

Having observed a relationship between red cell mass and arterial oxygenation in the smoking patients, we turned our attention to the mechanism of this response. The main site of erythrogenin release was identified as the kidney by Jacobson, Goldwasser, Fried and Plzak (1957), and several studies have shown that the isolated organ increases erythropoietic activity when perfused with hypoxic blood (Adamson and Finch, 1975). Our results show that the oxygen saturation of arterial blood appears to be a major determinant of erythropoietic activity, but results from Adamson (1970) and Miller and co-workers (1973) suggest that erythropoietic activity also depends upon the haemoglobin affinity for oxygen. This would suggest that it is the supply of oxygen to the tissue that is the most important determinant of erythrogenin release, and as oxygen supply depends upon several

factors in addition to arterial oxygen saturation, the relationship between red cell mass and arterial oxygen saturation is probably an oversimplification.

However, measurements of some of the other factors involved in oxygen delivery, such as $P50_{(7.4)}$, haemoglobin concentration, and pH, together with reasonable assumptions as to the oxygen combining power of blood, and the renal arterio-venous oxygen content difference, allowed us to estimate the tissue PO_2 within the kidney. As an arterio-venous oxygen content difference of ml/100 ml blood (which probably represents that across the renal medulla (Hollenberg, 1979)) gave the greater significant linear relationship, it may imply that this is the site where oxygen supply is critical for the release of erythrogenin. The oxygen supply to this site would be limited in those patients with an elevated carboxyhaemoglobin level, due to the greater oxygen affinity of haemoglobin and the lower oxygen capacity of the blood, and together these factors could account for the greater polycythaemic response in this group.

There remains some variability in response, which is still unexplained. Some of this variability may result from our assumption of a constant arterio-venous oxygen content difference (i.e. renal blood flow) in all the patients. In addition, investigations into oxygen supply during exercise (King, Cooke, Leitch and Flenley, 1973) and during sleep (Douglas, Calverley, Leggett, Brash, Flenley and Brezinova, 1979) have revealed increases in the severity of hypoxia in such patients, which may lead to an increase in red cell mass, producing a greater polycythaemic response than would be expected from results obtained in these patients, when awake and at rest, which may account for some of

the variation. However, despite some variability, the results of this study strongly suggest that carboxyhaemoglobin, produced by the combination of haemoglobin with carbon monoxide from cigarette smoke, plays an important role in enhancing the polycythaemic response to hypoxia in patients with chronic obstructive lung disease, by limiting the supply of oxygen to a site within the kidney. Although our results indicate the renal medulla as the most likely site of erythrogenin release, more direct measurements of PO_2 within this region are required to substantiate this hypothesis.

CHAPTER 6

Study of the effect of elevated COHb saturations on oxygen transport and exercise tolerance in chronic bronchitis and emphysema.

Subjects and experimental design

Nine patients (3 women, 6 men) with chronic bronchitis and emphysema were studied. The patients had severe airways obstruction ($FEV_{1.0}$ 0.35 - 1.90 L) and were hypoxaemic at rest arterial PO_2 43.0 - 59.0 mm Hg). Each patient was clinically stable when studied, and although some of the patients smoked, they were asked to refrain from smoking on the day of the study. All studies were carried out in the afternoon.

Venous blood samples were taken for determination of $P50_{(7.4)}$, 2,3-DPG concentration, haemoglobin concentration, carboxyhaemoglobin saturation and venous pH of whole blood and red cells, before the start of the study, and again after inhalation of 0.02% carbon monoxide over 15 minutes. Additional blood samples were taken for the determination of 2,3-DPG concentration and extra-and intra-cellular pH at the end of exercise both before and after the experimentally induced elevation of carboxyhaemoglobin. Arterial blood gases, 0_2 saturation and pH were also determined at the start of the study.

The exercise tolerance test employed was that of McGavin, Gupta and McHardy (1976). The subjects walked on the level, at their own pace, for 12 minutes, and the distance walked was recorded. During each walk, the patients received either air or oxygen at a flow rate of 2L/minute via nasal cannulae from a cylinder transported behind the patient. The patient was not aware of which gas was being administered.

Each patient was studied on two occasions and completed four walks on each occasion: breathing air then oxygen before elevation of carboxyhaemoglobin, and air then oxygen after elevation of carboxyhaemoglobin, with 20 minute rest periods between each walk. On the second occasion, the order of air and oxygen administration was reversed, both before and after carbon monoxide inhalation.

In 14 of the 18 studies, arterial oxygen saturation at rest and at the end of each exercise period was determined using the Hewlett Packard Ear Oximeter. This instrument gave a direct estimation of oxygen saturation at low carboxyhaemoglobin saturations, but at high carboxyhaemoglobin saturations, a correction factor had to be applied to the reading to give the oxygen saturation, which was expressed as a percentage of the total haemoglobin (SO_2T) (Douglas, Brash, Wraith, Calverly, Leggett, McElderry, Flenley, 1979). The correction factor is described by the equation:

$$\text{oximeter reading} = 0.987SO_2 + 0.628COHb - 1.465$$

where SO_2 = saturation of total haemoglobin with O_2

Using arterial saturation, arterial oxygen content (CaO_2) was calculated:-

$$CaO_2 = \frac{SO_2T}{100} \times O_2 \text{ Capacity}$$

where O_2 capacity is calculated as:

O_2 capacity = Hb (g/dl) x 1.34 where 1.34 = oxygen combining power

he femoral venous oxygen content (CvO_2) was then calculated
n the assumption that femoral arterio-venous oxygen content
ifference was either 7 or 10 ml oxygen/100 ml blood. This
emoral venous oxygen content was then expressed as
aturation (SvO_2);-

$$SvO_2 = \frac{CvO_2}{O_2 \text{ capacity}} \times 100$$

his saturation was then converted to femoral venous oxygen
ension using the oxygen dissociation curve determined for each
tient, and corrected to the venous pH.

The paired t-test was used to assess statistically
gnificant changes of mean $P50_{(7.4)}$, 2,3-DPG concentration,
id extra-and intra-cellular pH after elevation of carboxyhaemoglobin
d at the end of exercise. The changes in 12-minute walking distance
re analysed using the Wilcoxon Signed Rank test, as a normal
stribution of the population could not be assumed.

Results appertaining to the study of the effect of carboxy-haemoglobin on exercise tolerance

All patients were hypoxic at rest, breathing air (mean $Pa0_2$ = 52.0 ± 3.8 mm Hg), with moderate $C0_2$ retention (mean $PaC0_2$ = 45.2 ± 3.0 mm Hg) (Table 6; subjects 1 - 9). Each patient was clinically stable when studied, and had not smoked for 12 hours prior to the study. The mean initial COHb level was 3.0 ± 0.9% and rose to 12.6 ± 1.0% after exposure to carbon monoxide. As the CoHb level changed, the intial $P50_{(7.4)}$ value (mean 25.9 ± 0.8 mm Hg) decreased significantly ($P < 0.001$) to 24.7 ± 1.2 mm Hg, reflecting the increase in haemoglobin oxygen affinity caused by COHb.

The 12 minute walking distances for each patient, studied on two occasions, are shown in Table 7. Although the order of the two walking tests, breathing air and oxygen, were reversed on the second occasion, there was no significant difference between the distances walked when breathing air on those two occasions, nor the distances walked when breathing oxygen ($P > 0.10$), indicating the absence of a training effect, or patient fatigue.

Walking distances varied a great deal between subjects (range 168 metres - 991 metres), but allowing each subject to serve as his own control, changes in the distances walked while breathing the various gas mixtures can be seen. At low COHb levels the distance walked while breathing oxygen (mean 797 ± 174 metres) was found to be greater ($P < 0.20$) than that covered when breathing air (mean 767 ± 178 metres). Similarly, after elevation of CoHb levels, the average distance walked when breathing oxygen (771 ± 192 metres) was significantly greater ($P < 0.02$) than the average distance walked when breathing air (715 ± 201 metres) (Fig 24)

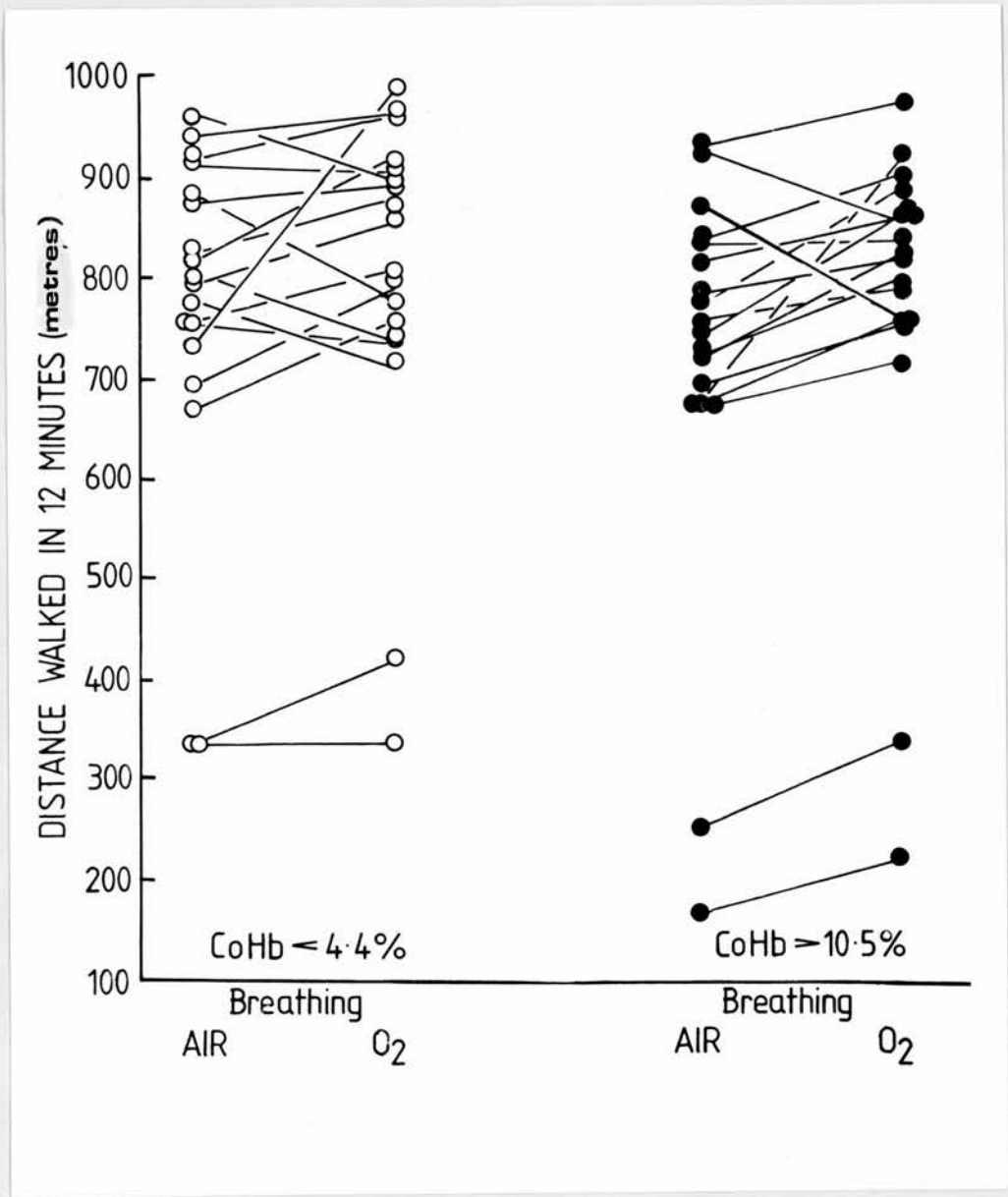


Figure 24

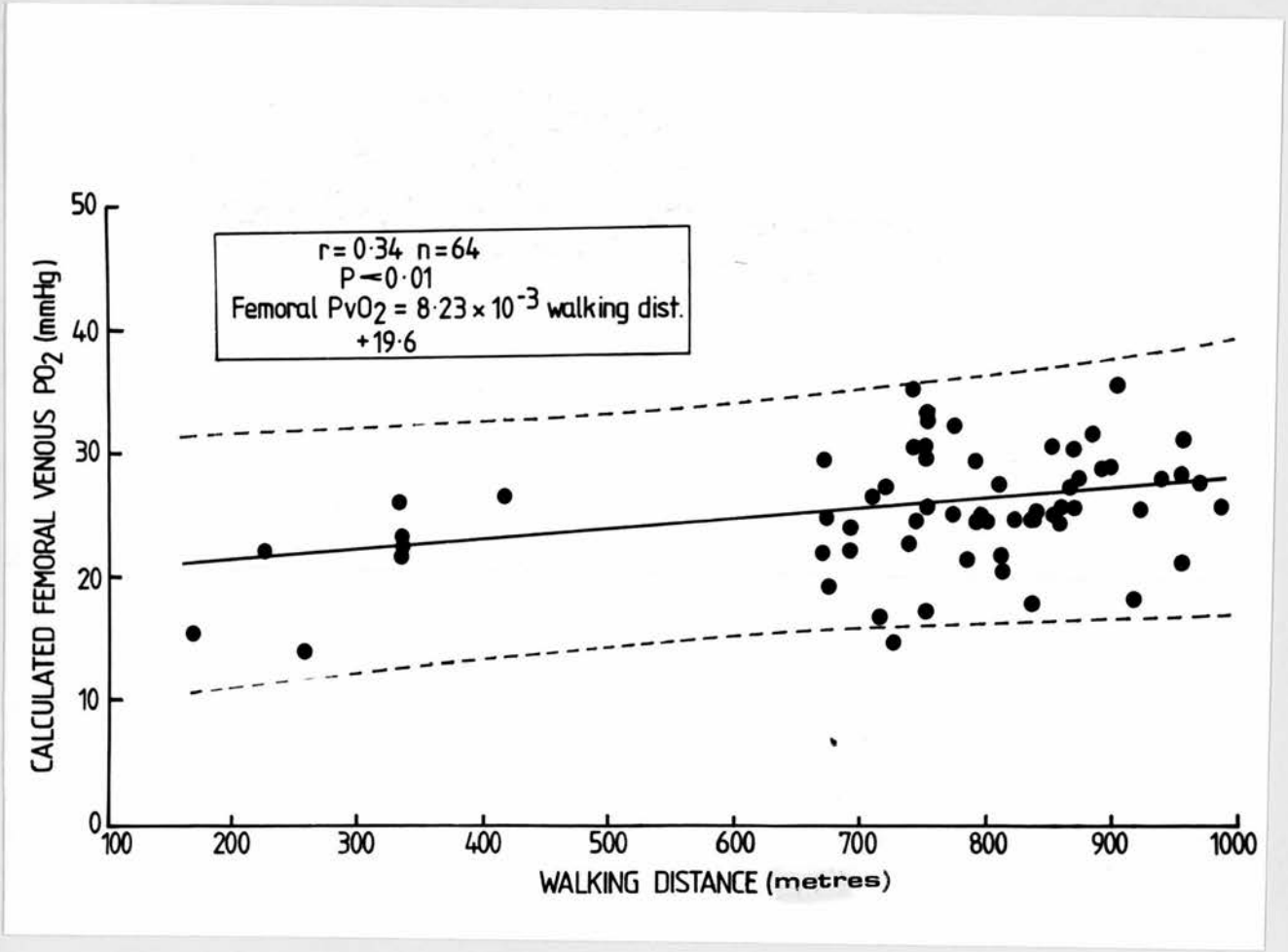
The 12-minute walking distances for 9 patients with chronic bronchitis, determined when the patients were breathing air and 02, before and after in vivo elevation of COHb levels on 2 occasions.

The distance walked when breathing air was significantly reduced ($P < 0.01$) after COHb levels had been increased, and similarly, the distance walked when breathing oxygen was reduced after elevation of COHb levels ($P < 0.10$).

The walking distances for each patient did not correlate with resting arterial PO_2 breathing air, nor with resting or post-exercise arterial oxygen saturation assessed by the Hewlett Packard ear oximeter (Tables 6,7). However, there was a significant correlation between the calculated levels of the femoral venous oxygen tension and walking distance when venous oxygen tension was calculated assuming an arterio-venous oxygen content difference of 7 mls/100 ml blood ($r = 0.34$, $P < 0.01$), and also when the venous oxygen tension was calculated assuming an arterio-venous oxygen content difference of 10 mls/100 ml blood ($r = 0.33$, $P < 0.01$) (Figs. 25 and 26).

However, as the 12 minute walking distance of subject 9 was markedly lower than that of the other subjects, the inclusion of this data may have weighted the relationship. Therefore, the relationship between femoral venous oxygen tension and walking distance was reassessed having omitted the results of subject 9. The femoral venous oxygen tension, calculated assuming an arterio-venous oxygen content difference of 10 mls/100 ml blood, showed a correlation with walking distance, significant at the 10% level ($r=0.25$) (Fig. 27), but there was no significant relationship between femoral venous oxygen tension, calculated assuming an arterio-venous oxygen content difference of 7 mls/100 ml blood, and walking distance (Fig. 28).

There was no significant change in mean resting 2,3-DPG concentration after elevation of COHb levels. However, at basal COHb concentration, the mean post-exercise 2,3-DPG



'igure 25

'emoral venous 0_2 tension, calculated assuming an arterio-venous 2 content difference of 7 ml 0_2/100 ml blood, is plotted against he 12-minute walking distances for each patient, measured when reathing air and 0_2, before and after in vivo elevation of COHb evels ($r = 0.34$, $P < 0.01$).

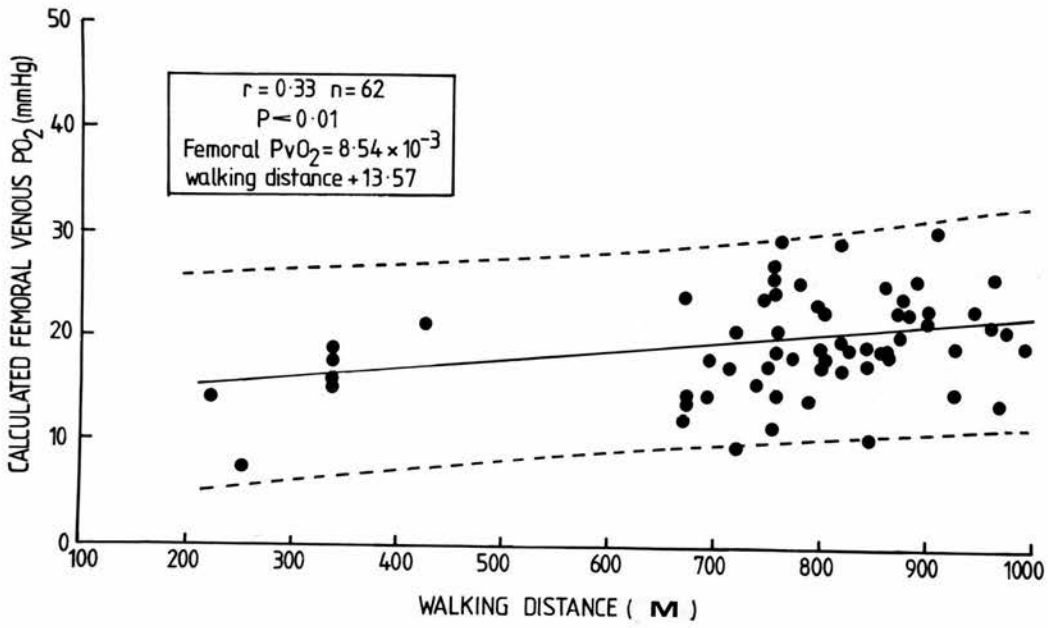
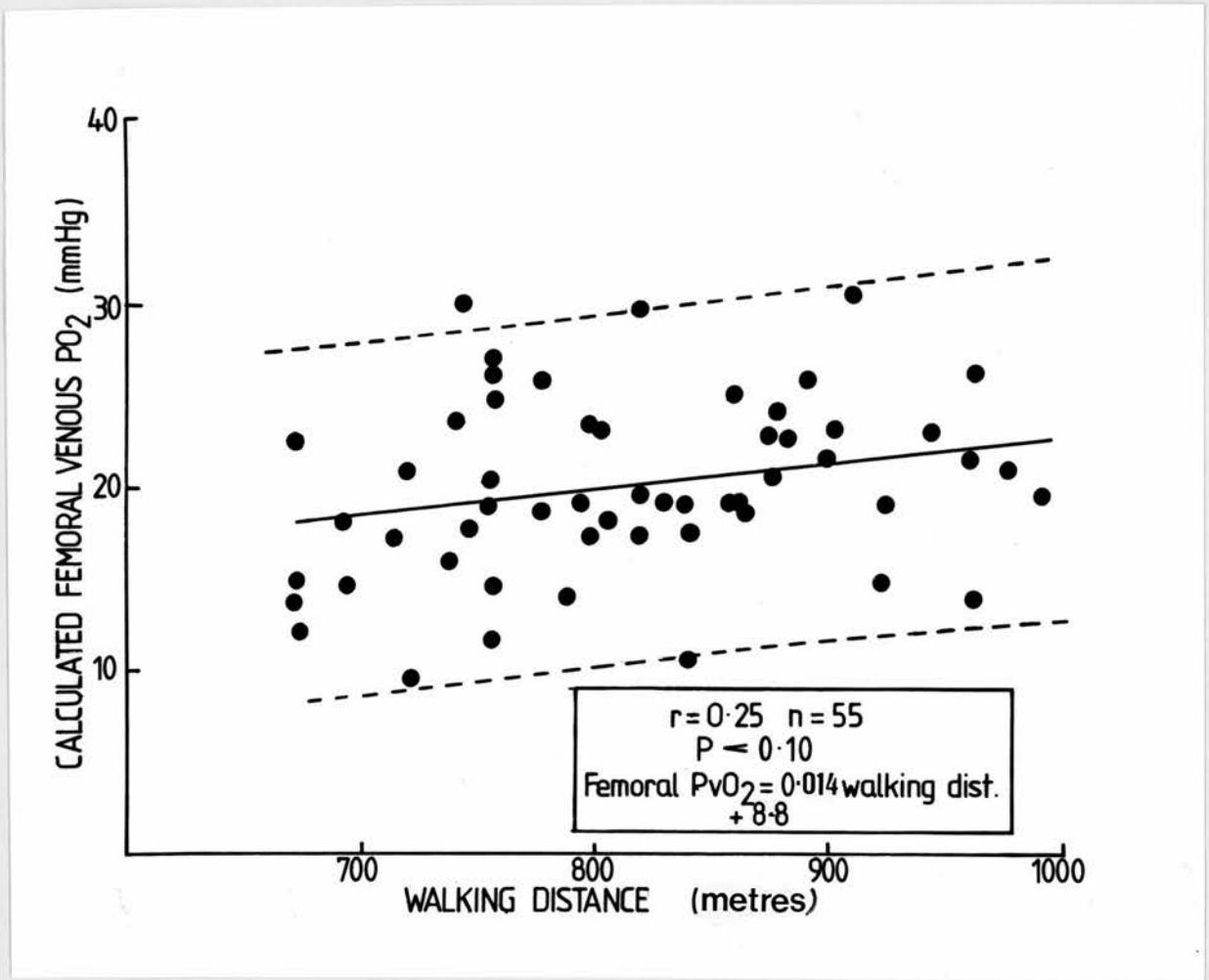


Figure **26**

Femoral venous O_2 tension, calculated assuming an arterio-venous O_2 difference of 10 ml O_2/100 ml blood, is plotted against the 12-minute walking distances for each patient, measured when breathing air and O_2, before and after in vivo elevation of CoHb levels ($r = 0.33$, $P < 0.01$).



igure 27

emoral venous O_2 tension, calculated assuming an arterio-venous
$_2$ content difference of 10 ml O_2/100 ml blood, is plotted against
he 12-minute walking distances for each patient (excepting patient
) when breathing air and O_2, before and after in vivo elevation
f COHb levels ($r = 0.25$, $P < 0.10$).

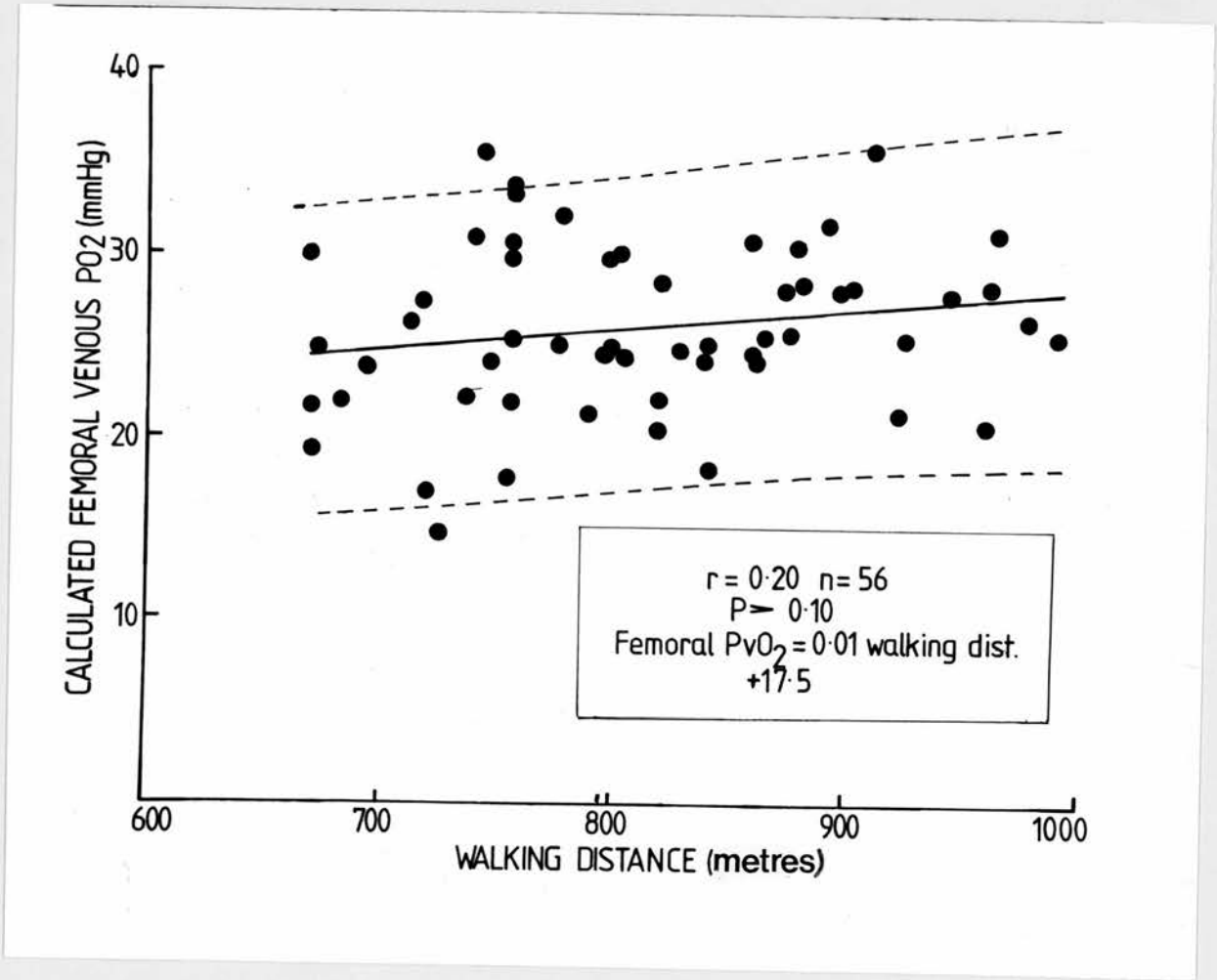


Figure **28**

Femoral venous O_2 tension, calculated assuming an arterio-venous O_2 content difference of 7 ml O_2/100 ml blood, is plotted against the 12-minute walking distances for each patient (excepting patient 9) when breathing air and O_2, before and after in vivo elevation of COHb levels ($r = 0.20$, $P > 0.10$).

concentration was significantly lower than the mean resting 2,3-DPG concentration, but this exercise-induced change in 2,3-DPG was not found after the COHb concentration had been raised(Table 8).

There was no significant change in mean resting extracellular and intracellular pH after elevation of COHb levels. However, after exercise at low COHb levels, there was a significant rise in mean extracellular and intracellular pH, although this post-exercise increase did not occur after elevation of COHb concentrations.(Table 8).

Discussion of results appertaining to the study of the effect of carboxyhaemoglobin on exercise tolerance

The results of this study confirm those of Leggett and Flenley (1977), who, using 12 minute walking distances as a measure of exercise tolerance, demonstrated the beneficial effect of oxygen on exercise capacity in patients with chronic bronchitis and emphysema. These authors, however, confined their investigations to patients with low carboxyhaemoglobin concentrations, and did not study the effect of elevated carboxyhaemoglobin concentrations on the exercise tolerance of patients when breathing air or oxygen.

Vogel and Gleser (1972), Aronow and Cassidy (1975) and Seppänen (1977) found a marked reduction in exercise tolerance in healthy subjects after elevation of carboxyhaemoglobin levels, and Aronow, Ferlinz and Glauser (1977) extended this observation to patients with chronic obstructive airways disease, when exercise tolerance was assessed by bicycle ergometer exercises.

The levels of carboxyhaemoglobin employed in our study were greater than those used by Aronow et al (1977), but as these higher levels corresponded to out-patient carboxyhaemoglobin levels observed in a number of patients who were smoking, it could not be considered excessive.

The 12 minute walking test (McGavin, Gupta and McHardy, 1976) provides a simple measure of disability in patients with chronic obstructive airways disease. It has been shown to provide more reproducible results that those obtained by other respiratory function, and exercise tolerance tests, which include measurement of minute ventilation, heart rate, and oxygen uptake at different

levels of exercise (Mungall and Hainsworth, 1979). However, the same authors found that there appears to be improvement in the performance of this test over the first two walks, but in this study we attempted to eliminate this effect by alternating the order of the walks breathing air and oxygen, on the two occasions that each patient was studied, so that if such a training effect was present, it would become apparent as a difference in the walking distances, while breathing the same gas mixture, on the two occasions. However, there was no significant difference between the walking distances on the two occasions while breathing the same gas mixtures, which suggests that the exercise test was free from the training effect reported by Mungall and Hainsworth (1979).

However, like all exercise tests, patient co-operation and commitment were vital to the accuracy of the results, and for this reason results from the very few patients who were unhappy to continue the studies to completion were rejected. Having taken these steps, we feel confident that the walking distances measured in this study accurately reflect the exercise tolerance of the patients.

The results of this study show that exercise tolerance does improve when oxygen is administered, both at normal carboxy-haemoglobin levels, and after inhalation of carbon monoxide to raise carboxyhaemoglobin levels by 10%. However, arterial oxygenation is not the major determinant of exercise tolerance, as indicated by the absence of a correlation between arterial oxygen saturation and 12 minute walking distance. The oxygen supply to the working muscles is the most important determinant of exercise capacity, and this depends upon the position and shape

of the oxygen dissociation curve, the oxygen content of the blood and leg blood flow. At low carboxyhaemoglobin levels, it is probable that arterial oxygenation reflects tissue oxygen supply, with the result that any increase in arterial oxygen saturation is accompanied by an increase in exercise tolerance, as shown by the patients in this study. However, elevation of carboxyhaemoglobin concentration impairs oxygen supply to the muscles regardless of the arterial oxygen tension or saturation, with the result that the distance walked when breathing air was reduced and the beneficial effects of oxygen on the 12 minute walking distances was effectively abolished, there being no significant change in the mean walking distance when breathing oxygen at high carboxyhaemoglobin concentration from the mean walking distance when breathing air at low carboxyhaemoglobin levels.

The presence of carboxyhaemoglobin lowers the oxygen carrying capacity of the blood, and also shifts the oxygen dissociation curve to the left, indicating increased affinity of haemoglobin for oxygen. This leftward shift of the dissociation curve appears to be unopposed by any immediate compensatory increase in 2,3-DPG or H^+ concentrations, as there was no significant change in the concentration of either of these haemoglobin ligands after acute elevation of carboxyhaemoglobin concentrations.

The leftward shift of the oxygen dissociation curve is most pronounced at the oxygen tensions present at the tissue level of exercising muscle (Vogel and Gleser, 1972), thus in order for oxygen extraction to remain unchanged, tissue oxygen tension must be lower than that present when carboxyhaemoglobin concentrations are low. From studies in normal subjects, Vogel and Gleser (1972)

demonstrated that after exposure to carbon monoxide, the lower oxygen delivery, resulting from the carbon monoxide-induced functional anaemia, was indeed compensated by tissue oxygen extraction to a lower venous oxygen tension, and suggested that when carbon monoxide exposure is combined with altitude, or disease-produced forms of hypoxia, their effect on tissue oxygen tension, and therefore exercise tolerance, would be additive, a suggestion supported by our results.

The relationship between the calculated femoral venous oxygen tension and the 12-minute walking distance observed in this study, suggests that muscle hypoxia is the limiting factor in exercise capacity in these patients, the existing hypoxia being aggravated by carbon monoxide inhalation from, for example, cigarette smoke. In addition, Flenley, Brash, Clancy, Cooke, Leitch, Middleton and Wraith (1979) suggested that the oxygen tension of exercising muscle may be important in stimulating hyperventilation during exercise. It is possible, therefore, that those patients with the lowest tissue oxygen tensions were hyperventilating maximally at an earlier stage of exercise, and so were unable to carry on exercising for the same length of time, due to ventilatory limitations, as those patients with a higher tissue oxygen tension.

CHAPTER 7

Subjects and experimental design

Blood from seven healthy men, all non-smokers, was studied in order to assess the magnitude of the CDH effect in normal subjects. Individual variation of the CDH effect was investigated in one subject studied on five occasions over a period of 20 days. Normal haematological status in these subjects was confirmed by measurement of $P50_{(7.4)}$ and 2,3-DPG and haemoglobin concentration.

Blood from 14 healthy women was similarly investigated to assess the normal CDH effect in women. Six of the women were smokers, but all refrained from smoking on the day of study to ensure carboxyhaemoglobin levels were low.

As initial results in some women suggested that the CDH effect varied greatly through the menstrual cycle, the women were divided into three groups to investigate this phenomenon further. Group A consisted of four women (age range 24 - 35 years, mean age 28 $\pm$ 5 years) who were not taking oral contraceptives. All were studied at menstruation and as close as possible to ovulation, as judged by body temperature. Normal haematological status was confirmed by measurements of $P50_{(7.4)}$, 2,3-DPG and haemoglobin concentration. Investigations were repeated in two of the four subjects.

Group B consisted of eight women (age range 18 - 25 years, mean age 22 $\pm$ 3 years) who were established on a course of oral contraception* for between 12 and 48 months. All were studied at menstruation, usually between days 3 - 5 of the 7-day "pill-free" phase of the cycle, and again between days 14 - 18 of the cycle. In addition to the measurements of $P50_{(7.4)}$, 2,3-DPG and haemoglobin concentrations carried out in all subjects, estimations of plasma haemoglobin, met-haemoglobin, reduced glutathione and ferritin were also

* oral contraceptive: 30μg ethinyloestradiol, 0.15 mg D-norgestrel

carried out in three women (by staff of the Department of Haematology, Royal Infirmary of Edinburgh), and glycosylated haemoglobin levels were also determined in one subject for two consecutive months (by staff of the Department of Clinical Chemistry, Royal Infirmary of Edinburgh). All measurements of the CDH effect in these subjects, with the exception of one subject, were repeated at least once. Using blood from one subject in group B who showed marked variation in the CDH effect through the menstrual cycle, the CDH effect was determined simultaneously in whole blood and also in washed red blood cells resuspended in physiological saline containing fatty acid-free albumin and bicarbonate ions. This procedure was carried out at menstruation and mid-cycle for two months, to determine whether the changing CDH effect is a property of red blood cells or plasma.

Group C consisted of two women (aged 21 and 29 years) who were starting a course of oral contraception. Both were studied at menstruation immediately prior to starting oral contraception, and at mid-cycle and menstruation for the first two months of oral contraceptive treatment. In both subjects $P50_{(7.4)}$, 2,3-DPG and haemoglobin concentrations were determined, together with plasma haemoglobin, methaemoglobin, reduced glutathione and ferritin. Glycosylated haemoglobin levels were determined in one subject.

Statistical comparison of mean values between the groups of women were carried out using the unpaired 't' test.

The CDH effect was also investigated in 10 patients (3 post-menopausal women and 7 men) with chronic bronchitis and emphysema, aged 54 - 70 years. All patients had severe

airways obstruction (mean $FEV_{1.0}$ 0.6 ± 0.29 L) and were hypoxaemic at rest (mean arterial PO_2 51.5 ± 4.2 mm Hg), but were free from infection and in a stable clinical state when studied. Although several patients smoked, they were asked to refrain from smoking on the day of the study.

Each patient was studied twice, venous blood being taken on each occasion for determination of the CDH effect, $P50_{(7.4)}$, 2,3-DPG and haemoglobin concentrations at a low carboxyhaemoglobin concentration, and again after in vivo carboxyhaemoglobin saturation had been raised by 10%.

Statistical comparisons of mean values of the CDH effect at low carboxyhaemoglobin saturations and high carboxyhaemoglobin saturations were carried out using the paired 't' test, and comparison between the mean CDH effect of normal subjects and that of the patients was carried out using the unpaired 't' test.

Results appertaining to the investigation of the C.D.H. effect

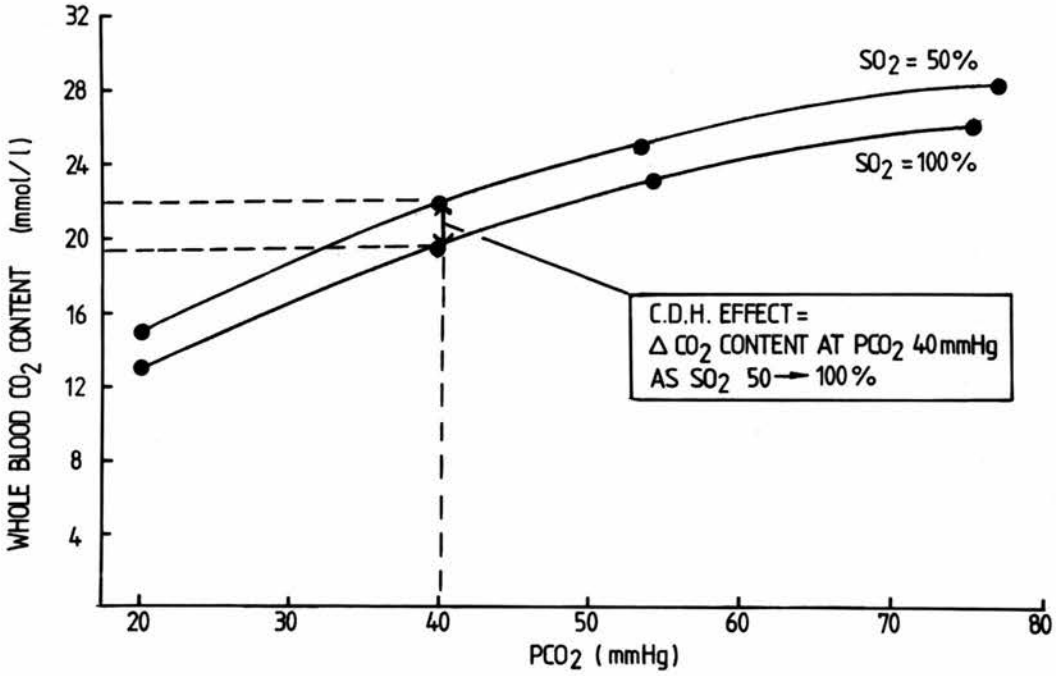
For each subject, carbon dioxide dissociation curves were constructed at haemoglobin oxygen saturations of 50 and 100%, as shown in Figure 29. From these curves, the C.D.H. effect was obtained as the difference in carbon dioxide content between the two curves at a carbon dioxide tension of 40 mm Hg and expressed as mmoles of carbon dioxide per litre of whole blood. Expressed in this way, however, the C.D.H. effect did not relate to a constant change in whole blood oxygen content, as haemoglobin concentration varied markedly between subjects. This was overcome by calculating the change in oxygen content accompanying the partial reduction of oxyhaemoglobin, and expressing the C.D.H. effect as the change in CO_2 content per change in O_2 content at PCO_2 of 40 mm Hg.

a) Results from normal male subjects

Results obtained in seven normal healthy men are shown in Table **9** . In each subject, COHb levels were below 3.0% and the mean $P50_{(7.4)}$ and 2,3-DPG concentration were within the normal range. The C.D.H. effect in these men (mean value 0.34 ± 0.03 mM CO_2/mM change in O_2) showed little variation between subjects, and maintained a reasonably constant value in one man studied on five occasions over a period of 20 days (mean 0.32 ± 0.03 mM CO_2/mM change in O_2, range 0.29 - 0.35 mM CO_2/mM change in O_2).

b) Results from normal female subjects

In women, the situation proved more complex. Those women in group A, who were not taking an oral contraceptive,



'igure 29

'hole blood CO_2 dissociation curves at oxyhaemoglobin saturations
'f 50% and 100% in a normal male subject, showing the CDH effect
s determined in this study, and defined as the change in CO_2 content
t PCO_2 40 mm Hg accompanying an oxyhaemoglobin saturation change
f 50%.

showed little variation in the C.D.H. effect between subjects, and the mean value at menstruation (mean 0.35 ± 0.07 mM CO_2/mM change in O_2) did not differ significantly from that at the mid point of the menstrual cycle (0.34 ± 0.05 mM CO_2/mM change in O_2). In vitro $P50_{(7.4)}$, together with haemoglobin and 2,3-DPG concentrations were normal, and did not alter significantly through the menstrual cycle (Table **9**).

c) Results from normal females taking oral contraceptives

The mean mid-cycle C.D.H. effect for women in group B, who were established on a course of oral contraception, was similar to that of group A (mean value 0.34 ± 0.04 mM CO_2/mM change in O_2), but at menstruation, the C.D.H. effect in group B (mean value 0.22 ± 0.04 mM CO_2/mM change in O_2) was significantly lower ($P < 0.001$) than that in group A (Fig. 30). 2,3-DPG and haemoglobin concentrations were within the normal range and did not alter significantly during the menstrual cycle, but in vitro $P50_{(7.4)}$ was significantly higher ($P < 0.05$) at menstruation (mean value 27.4 ± 1.8 mm Hg) than at mid-cycle (mean 26.8 ± 1.6 mm Hg) in these women (Table **9**).

The concentrations of reduced glutathione, methaemoglobin, plasma haemoglobin and ferritin, determined in three women from group B, did not alter significantly during the menstrual cycle. In addition, the concentration of glycosylated haemoglobin determined in one woman at menstruation and mid cycle over two consecutive months, did not vary, although the C.D.H. effect in this subject did alter through the cycle (Table **10**).

The simultaneous determination of the C.D.H. effect using whole blood and resuspended red cells from one subject taking an oral contraceptive, showed no difference between the C.D.H.

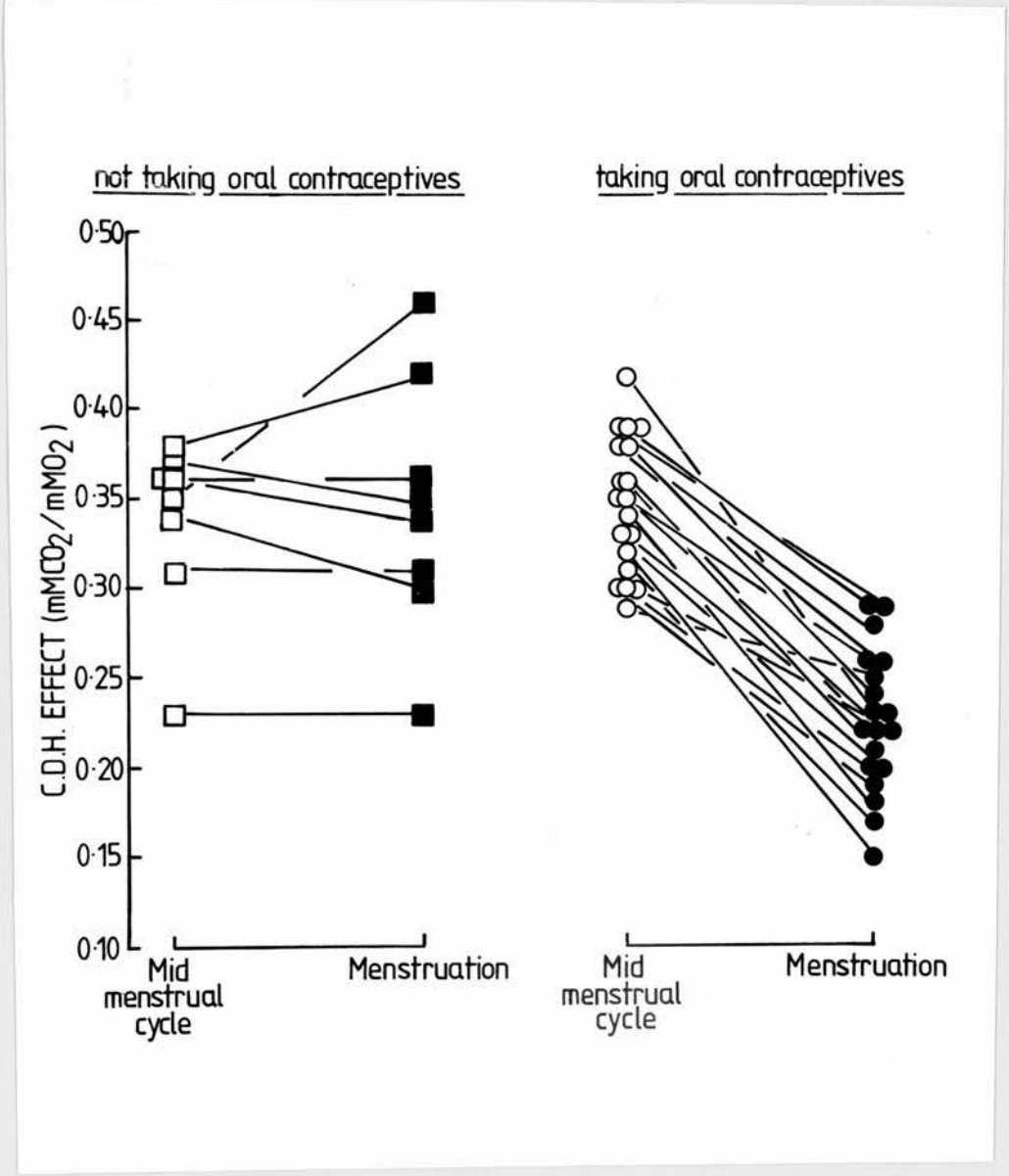


Figure 30

The CDH effect determined at menstruation and mid-menstrual cycle in women who were not taking oral contraceptives (■,□) and women taking the combined oestrogen-progesterone contraceptive pill (●,○).

effect determined in whole blood and that determined using resuspended red cells. In this subject the C.D.H. effect, determined on two separate occasions at menstruation was 0.23 and 0.29 mM CO_2/mM change in O_2 in whole blood, and 0.22 and 0.27 mM CO_2/mM change in O_2 in resuspended red cells. At mid cycle, the C.D.H. effect determined in whole blood was 0.39 and 0.40 mM CO_2/mM change in O_2, and in resuspended red cells was 0.40 and 0.42 mM CO_2/mM change in O_2.

d) Results from normal females beginning a course of oral contraceptives

The C.D.H. effect, together with $P50_{(7.4)}$ and concentrations of 2,3-DPG and haemoglobin determined in two women immediately before and for two months after beginning a course of oral contraception are shown in Table **11** . Subject 18 had a normal C.D.H. effect immediately before and 14 days after beginning oral contraception, but after one month, the C.D.H. effect was markedly lower than the pre-treatment value. At mid cycle during the second month, the C.D.H. effect returned to normal, but decreased again at menstruation at the end of the second month (Fig.**31**).

In subject 17, the C.D.H. effect determined immediately prior to beginning the course of oral contraception was slightly higher than normal, but fell to within the normal range after 14 days of oral contraception. There was no reduction in the C.D.H. effect at menstruation in this subject during the first month, and it remained essentially unchanged at mid cycle in the second month. However, at the end of the second month a reduction in the C.D.H. effect was observed (Fig.**31**).

The C.D.H. effect in both these subjects bore no relationship to levels of reduced glutathione, methaemoglobin, plasma haemoglobin, nor ferritin. Similarly, glycosylated haemoglobin

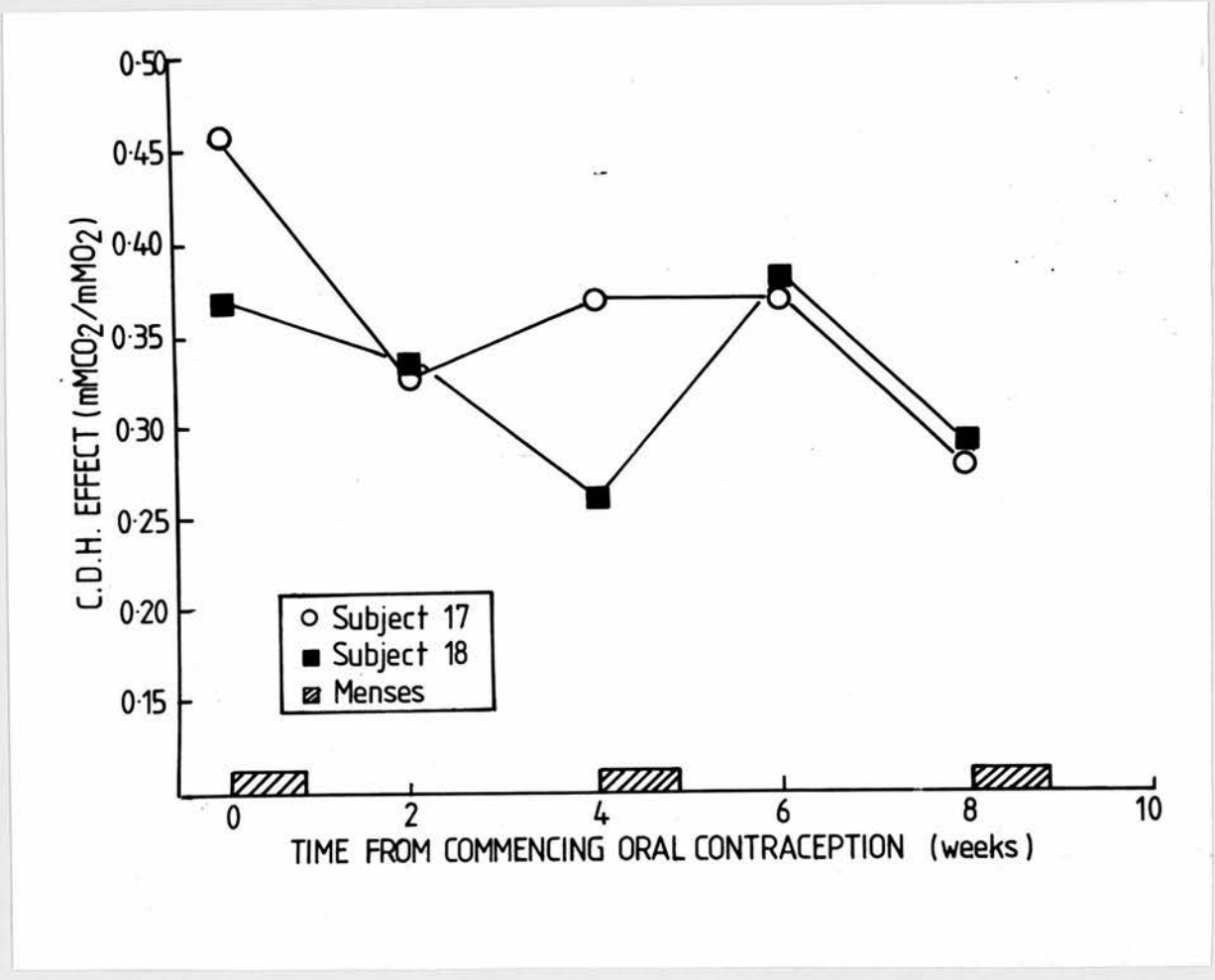


Figure 31

The CDH effect determined in two women immediately before, & at two week intervals, for two months after beginning a course of oral contraception, showing the development of cyclical variation in the CDH effect.

levels, measured in subject 17, did not correlate with the C.D.H. effect (Table **12**).

e) Results from patients with chronic bronchitis

In ten patients with chronic bronchitis and emphysema, all of whom were hypoxaemic when breathing air at rest (Table **4**), the mean C.D.H. effect measured on two occasions when carboxyhaemoglobin levels were low was 0.30 ± 0.07 mM CO_2/mM change in O_2, a value which did not differ significantly from that in normal subjects. However the patients showed a greater range of values for the C.D.H. effect than the normal subjects (range 0.18 - 0.50 mM CO_2/mM change in O_2) (Table **9**), and with the exception of only one patient (**3**), the values remained reasonably constant over the two measurements.

After in vivo elevation of carboxyhaemoglobin levels, there was no significant change in the mean C.D.H. effect (0.33 ± 0.08 mM CO_2/mM change in O_2) from that observed at low carboxyhaemoglobin levels, but again the values were scattered over a wide range (range 0.21 - 0.49 mM CO_2/mM change in O_2).

In addition the patients demonstrated a higher carbon dioxide combining power (defined as whole blood carbon dioxide content at PCO_2 of 40 mm Hg, when fully saturated with oxygen) than the normal subjects, resulting from the prevailing high PCO_2 in these patients.

Discussion of results appertaining to the investigation of the CDH effect

The CDH effect, as determined in this study, is the result of two mechanisms: a) the change in pH accompanying oxygenation, which alters the concentration of bicarbonate, and b) the oxylabile binding of carbon dioxide to haemoglobin as carbamate. At pH 7.40 and PCO_2 40 mm Hg, the change in bicarbonate concentration on oxygenation accounts for 60% of the CDH effect, with the change in carbamate concentration accounting for only 40% of the CDH effect (Klocke, 1973).

In the construction of CO_2 dissociation curves, pH was allowed to change with PCO_2, mimicking a state of acute respiratory acid base imbalance (in vitro). The results of Klocke (1973) suggest that under these conditions, the CDH effect remains reasonably constant over the PCO_2/pH range used in our study, although the relative contributions of changes in carbamate and bicarbonate concentrations to the CDH effect will vary with PCO_2 and pH. As the pH increases, the formation of carbamate in reduced haemoglobin increases, with the result that on oxygenation at a pH of 7.6, the change in carbamate concentration is greater than that occurring at a lower pH. This increased dissociation of carbamate at the higher pH value allows greater buffering of hydrogen ions which reduces the change in bicarbonate concentration accompanying oxygenation, thereby reducing its relative contribution to the CDH effect. At lower pH values the reverse is true, with the change in bicarbonate concentration contributing most to the CDH effect, but the overall change in carbon dioxide content on oxygenation remains reasonably constant.

The CDH effect determined in this study, and expressed as the change in carbon dioxide content per change in oxygen

content, could not be compared directly with estimates of the CDH effect made by previous authors, for, despite being expressed in the same way, previous determinations involved the complete reduction of oxyhaemoglobin, whereas in this study, oxyhaemoglobin was reduced by only 50%. In order to compare our results with those of others, it was necessary to demonstrate that the change in whole blood carbon dioxide content accompanying 50% reduction of oxyhaemoglobin is half of that accompanying complete reduction. Ferguson (1936) and Baumann, Bauer and Haller (1975) suggest that at constant PCO_2 the carbamino binding of carbon dioxide to haemoglobin is linearly related to oxygen saturation, but Garby, Robert and Zaar (1972) suggest this is not so. However, none of these authors determined the relationship pertinent to this discussion, that is, the relationship between whole blood carbon dioxide content (which includes bicarbonate and carbamate) and oxygen saturation. We investigated this relationship by equilibrating aliquots of whole blood with gas mixtures containing 6% CO_2 and various concentrations of O_2, designed to produce a range of oxyhaemoglobin saturations. The pH of the samples was allowed to change with saturation in order to mimic the conditions of the main experiments. The results shown in Fig.**32** indicate that the relationship between carbon dioxide content and oxygen saturation is linear, and also, that, for comparison purposes, the change in carbon dioxide content determined as oxyhaemoglobin is reduced to 50%, closely approximates half of that occurring when oxyhaemoglobin is fully reduced. Thus, when expressed per change in oxygen content, the CDH effect determined in this study is equivalent to that,

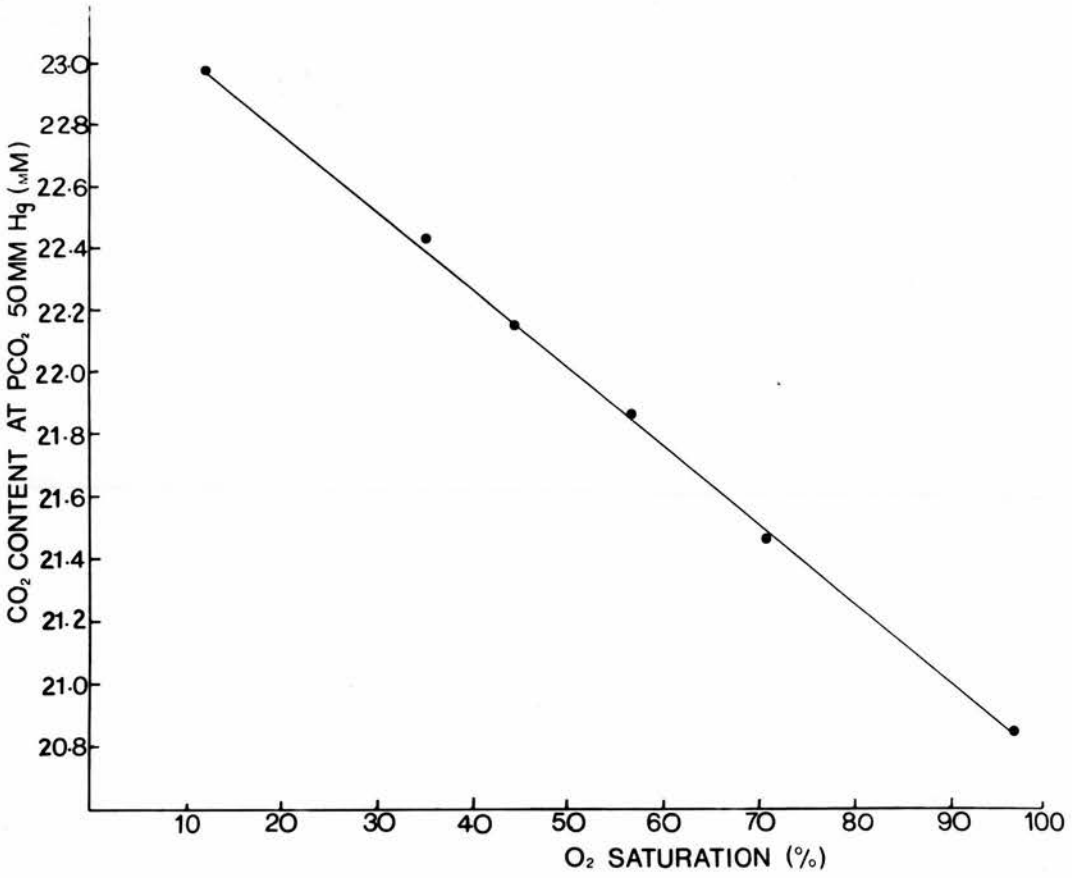


Figure 32

The relationship between whole blood CO_2 content and O_2 saturation at PCO_2 50 mm Hg, indicating that CO_2 content varies linearly with O_2 saturation.

expressed in a similar way, in other studies.

The normal CDH effect

The mean CDH effect determined in normal men (0.34 ± 0.03 mM CO_2/mM change in O_2) and normal women who were not taking oral contraception (Group A) (0.34 ± 0.05 mM CO_2/mM change in O_2 at mid menstrual cycle and 0.35 ± 0.07 mM CO_2/mM change in O_2 at menstruation) compares favourably with results obtained by others. Christiansen, Douglas and Haldane (1914), using whole blood, obtained results which, when expressed as above, gave a value of 0.34 mM CO_2/mM change in O_2, and Ferguson (1936) found the CDH effect in haemoglobin solutions was 0.35mM CO_2/ mM change in O_2.

The CDH effect in patients with chronic bronchitis

The CDH effect in patients with chronic obstructive airways disease (mean 0.30 ± 0.07 mM CO_2/mM change in O_2) is comparable with that in the normal subjects, and is similar to that determined by Lenfant, Ways, Aucutt and Cruz (1969) in a similar group of patients with chronic airways obstruction.

Using the carbon dioxide dissociation curves determined in patients with chronic bronchitis, the rise in PCO_2 accompanying oxygenation at a constant whole blood CO_2 content can be estimated. From Fig.29 it can be seen that an increase in oxygen saturation from 50% to 100% would increase the PCO_2 by 6 - 8 mm Hg, if CO_2 content remained constant. However, it has been reported that in vivo, the slope of CO_2 dissociation curves is less steep than it appears in vitro due to the redistribution of bicarbonate between intra- and extravascular spaces in vivo (Roos and Thomas, 1967). This would suggest a greater rise in PCO_2 accompanies oxygenation at a constant CO_2 content in vivo. However, the extravascular fluid in the pulmonary circulation is

negligible in comparison to the amount of circulating blood, so that the in vitro CO_2 dissociation curve could be considered to be the in vivo one, and the rise in PCO_2 on oxygenation in the lungs could be predicted from the in vitro curves.

The CDH effect, together with the reduction in ventilation on removal of the hypoxic stimulus by oxygen administration, may lead to hypercapnia in some patients. Data from Flenley, Franklin and Millar (1970) indicated an average rise in PCO_2 of 16 mm Hg as oxygen saturation increased from 86% to 100%. As CO_2 content is linearly related to oxygen saturation, it can be assumed that the CDH effect accompanying this rise in oxygen saturation of 14%, will be 28% of the CDH effect accompanying a rise in oxygen saturation of 50%, which has been determined in this study. Thus, as the oxygen saturation is raised from 86% to 100%, the theoretical rise in PCO_2 resulting from the CDH effect would be 2 mm Hg, accounting for only 12.5% of the 16 mm Hg rise in PCO_2 observed in these patients by Flenley et al (1970), the remainder presumably resulting from the removal of the hypoxic ventilatory drive.

In addition, our results indicate that an increase in carboxyhaemoglobin concentration, in vivo, does not alter the CDH effect accompanying a constant change in oxygen saturation, if oxygen saturation is expressed as a percentage of total haemoglobin (i.e. SO_2T), which suggests that the rise in PCO_2 accompanying oxygenation will be constant whether the patient is a smoker or a non-smoker.

The CDH effect in women taking oral contraceptives (Groups B & C)

Variation in the magnitude of the CDH effect throughout the menstrual cycle develops quickly following the start of oral

contraception, the characteristic decrease in the magnitude of the CDH effect at menstruation appearing within the first two months of treatment.

Because we were able to demonstrate variation of the CDH effect in both whole blood and washed red blood cells, it appears that this cyclical variation is produced by a change in the properties of the red cells, as distinct from a change in the properties of the plasma. It is unlikely that oestrogens and progestagens are directly responsible for these changes within the red cells, for changes in the concentration of these substances through the menstrual cycle in women who are taking oral contraceptives are negligible when compared with the changes which occur in normal ovulating women, and even during the early follicular phase of the menstrual cycle, levels of oestradiol in normal women are three times those in oral contraceptive users (Kjeld, Puah and Joplin, 1976). Furthermore, it is unlikely that sex steroids can penetrate the red cell membrane due to their large molecular dimensions. It appears more reasonable to suggest that chronic low levels of exogenous oestrogens and progestagens induce secondary changes, and it is these changes which influence the red cell. However, these changes within the red cell have not been identified despite a number of pertinent investigations:-

a) the consistency of 2,3-DPG and glycosylated haemoglobin concentrations throughout the menstrual cycle suggests that neither of these substances were responsible for the change in the CDH effect, despite having the ability, in theory, to block the formation of carbamate compounds in the haemoglobin molecule, as both 2,3-DPG, and the hexose moiety in glycosylated haemoglobin bind to the same α-amino groups as carbon dioxide.

b) levels of plasma haemoglobin, reduced glutathione, methaemoglobin and ferritin were monitored in a vain attempt to relate changes in the CDH effect to any changes in the red cell membrane integrity, the redox state of the haemoglobin iron, or the turnover rate of haemoglobin, but again, no relationship emerged.

c) the change in base excess which accompanies reduction of oxyhaemoglobin did not differ throughout the menstrual cycle, indicating that there was no change in the magnitude of the pH-shift accompanying the reduction of oxyhaemoglobin, and this could not therefore have influenced the pH-dependent formation of carbamate.

Several authors have demonstrated an increase in ventilation after administration of progesterone (Hasselbalch, Gammeltoft, 1915; Bohelmann, Rother, 1924; Lyons, 1969). Although the mechanism of this action is not certain, the hyperventilation produced, leads to a respiratory alkalosis, which is compensated by a fall in bicarbonate concentration. At menstruation, 3 - 4 days after oral contraceptive administration has stopped, ventilation is restored to normal, and bicarbonate concentration rises again due to renal reabsorption. The in vitro consequence of these changes is that at mid cycle, the blood will be more acidotic when equilibrated with a given PCO_2 than it will be at menstruation. However, as shown by the work of Klocke (1973) (Fig. 7), changes in pH over the range 7.3 - 7.6 have little effect on the overall CDH effect, and it is unlikely that this acid base imbalance could account for the large changes in the CDH effect observed in this study.

Thus, we have not been able to explain the mechanism of the change in the CDH effect which occurs at the time of

menstruation in women taking combined oestrogen/progesterone oral contraceptives.

Comparison of measured and calculated values for whole blood CO_2 content

From measurements of whole blood CO_2 content made using the I.R.G.A., we were able to assess the accuracy of the formula of Visser (1960) for predicting whole blood CO_2 content, and the change in CO_2 content accompanying the oxygenation of haemoglobin. Using PCO_2 and pH values obtained during the construction of CO_2 dissociation curves for both the normal subjects and the patients with chronic airways obstruction, plasma CO_2 content was estimated by the Henderson-Hasselbalch equation, assuming pK' of 6.1:-

$$[CO_2] = 0.03\ PCO_2\ (1 + 10^{\ pH\ -6.1})$$

and this, together with values for haemoglobin concentration, oxygen saturation, and capacity were then substituted into Visser's equation:-

$$\text{Blood } CO_2 \text{ content} = \text{Plasma } CO_2 \text{ content } \left(1 - \frac{0.0215 \times O_2 \text{ capacity}}{(2.244 \times 0.422\ SO_2)(8.74 - pH)}\right)$$

By repeating these calculations for each subject, using data at two levels of oxygen saturation, the change in CO_2 content accompanying oxygenation could be calculated, although this value was not necessarily the CDH effect as defined in this study, as CO_2 tensions at the two levels of oxygen saturation were not always equal.

The whole blood CO_2 contents (expressed as mmol/l) measured by the IRGA and calculated using the formula of Visser are compared in Fig.**33.** The data from the normal subjects deviates slightly from the line of identity, the calculated values being lower than the measured values over the range 17.5 - 22.5 mmol/l. Data from the patients, however, shows greater deviation from the line of identity over the range 18.5 - 26.0 mmol/l, with the calculated CO_2 content being significantly lower than the measured CO_2 content ($P < 0.001$).

When the formula was used to estimate the change in CO_2 content accompanying a change in oxygen saturation, a comparison with the measured values showed that the data was evenly distributed about the line of identity, but individual results show wide variation between the calculated and measured values (Fig. **34**).

It appears, therefore, that the formula of Visser is adequate to provide an approximate estimation of whole blood CO_2 content in normal subjects only; most similar calculations in patients with chronic bronchitis being underestimated. Furthermore, the wide individual variations observed between the calculated and measured values for changes in CO_2 content accompanying changes in oxygen saturation, in both normal subjects and patients with chronic bronchitis, suggest that the formula would be unsatisfactory for estimating the CDH effect, although the least squares regression line for the data lies close to the line of identity.

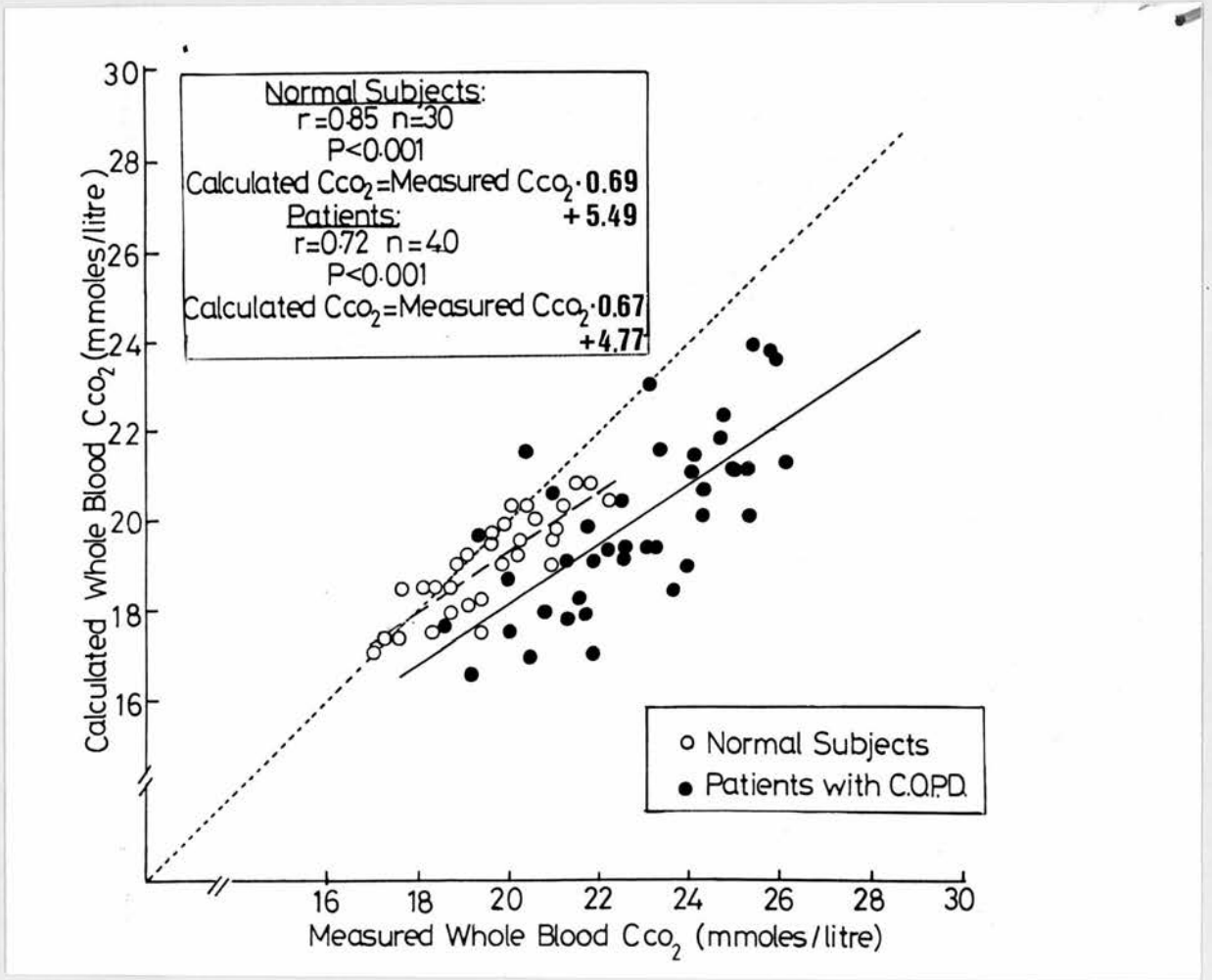


Figure 33

A comparison of calculated whole blood CO_2 content (calculated using the formula of Visser (1960)) with that measured using the I.R.G.A. in normal subjects (o) ($r = 0.85$, $P < 0.001$), and patients with chronic bronchitis (•) ($r = 0.72$, $P < 0.001$).
(-------) represents the line of identity.

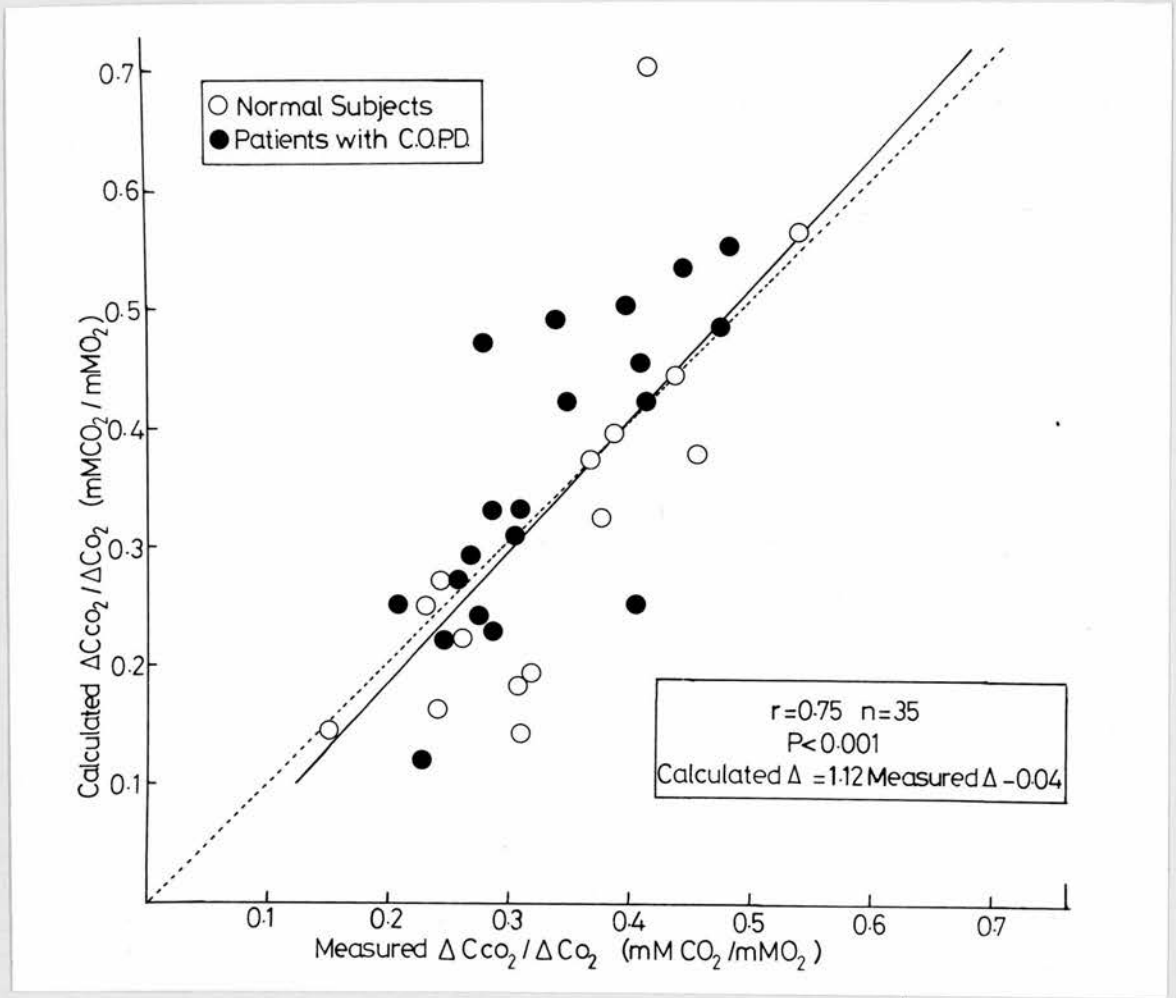


Figure 34

A comparison of the calculated change in whole blood CO_2 content (calculated using the formula of Visser (1960)) with the measured change occurring as oxygenated haemoglobin is reduced to 50% oxygen saturation in normal healthy subjects (o) and patients with chronic bronchitis (●) ($r = 0.75$, $P < 0.001$).

CHAPTER 8

Conclusions

In final answer to the questions posed in Chapter II therefore:-

1. Long term oxygen therapy does not alter the position or shape of the oxygen dissociation curve of the blood, nor does it affect the mean corpuscular haemoglobin concentration, or arterial blood gas and pH values obtained with the patient breathing air. However, haemoglobin concentration, packed cell volume and 2,3-DPG concentration decreased in those patients receiving oxygen therapy after only one year of treatment, these lower levels being maintained for the remainder of the study. No corresponding changes were observed in the control group patients.

2. The degree of secondary polycythaemia associated with chronic bronchitis is related to the smoking habits of the patients, and the correlation between renal medulla PO_2 and red cell mass ($r = -0.51$, $P < 0.001$) suggests that hypoxaemia in this region of the kidney may activate the erythropoietic response to hypoxia.

3. Exercise tolerance is significantly impaired following acute elevation of carboxyhaemoglobin concentration in patients with chronic bronchitis and emphysema. The exercise tolerance in such patients is related to tissue oxygenation, and is increased when the oxygen supply to the working muscles is increased. However, as carbon monoxide displaces oxygen from combination with haemoglobin, the benefit of oxygen administration to patients with elevated carboxyhaemoglobin concentrations is limited by the lower oxygen carrying capacity of the blood, with the result that exercise tolerance in such patients is lower than that in patients with normal carboxy-haemoglobin concentrations, despite comparable arterial PO_2 levels.

4. The formula of Visser (1960) appears suitable to provide an approximate indication of whole blood CO_2 content in normal subjects, but it is unsuitable for the calculation of CO_2 content in patients with chronic bronchitis, most of the calculated values being lower than the measured values. When the formula is used to assess the change in CO_2 content accompanying a change in O_2 content, marked individual variations between the measured and calculated values suggest that the formula is unsuitable for the estimation of the CDH effect.

5. The magnitude of the CDH effect determined in patients with chronic bronchitis was similar to that determined in normal subjects, and calculations based on these measurements suggest that only about 12% of the overall rise in PCO_2, observed in some patients when arterial oxygenation is increased, is a result of the CDH effect, the remainder resulting from the removal of the hypoxic ventilatory drive.

6. The CDH effect in patients with chronic bronchitis is not altered by elevation of carboxyhaemoglobin concentration in vivo, indicating that cigarette smoking in such patients would have no effect on the CDH effect,

7. In normal women the CDH effect remains constant throughout the menstrual cycle. In women taking the combined oestrogen-progesterone contraceptive pill, the CDH effect mid-way through the cycle is comparable with that in normal women, but at menstruation, the magnitude of the CDH effect falls, the mean value at menstruation being only 65% of the mean mid-cycle value.

8. The biochemical mechanisms responsible for the variation in the CDH effect through the menstrual cycle in women taking oral contraceptives remain obscure.

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TABLES

Table 1a

Arterial blood gas and pH data, $F.E.V._{1.0}$, R.C.M. and $\overline{P.A.P.}$ results in control group patients at start of study.

Subject	Age (yr.)	Arterial PO_2 (mm Hg)	Arterial PCO_2 (mm Hg)	Arterial pH	$F.E.V_{1.0}$ (l)	R.C.M. (ml/kg)	$\overline{P.A.P.}$ (mm Hg)
1 F	61	58	57	7.39	0.20	29	35
2 M	63	60	46	7.41	1.35	25	25
3 F	62	53	57	7.40	0.45	32	41
4 M	58	53	54	7.39	0.50	31	32
5 F	55	47	64	7.33	0.75	48	28
6 F	55	57	50	7.39	0.70	33	18
7 M	68	44	56	7.32	0.45	43	40
8 F	58	47	52	7.43	0.45	28	43
9 M	53	55	55	7.39	0.55	35	47
10 M	63	53	53	7.37	0.65	33	36
11 M	54	50	67	7.39	0.70	42	54
12 M	42	54	49	7.41	0.41	43	60
13 M	48	57	56	7.44	0.35	29	31
Mean ± S.D.	56.9 ± 6.9	52.9 ± 4.8	55.1 ± 5.7	7.39 ± 0.03	0.58 ± 0.28	34.7 ± 7.1	37.7 ± 11.6

Table 1b

Arterial blood gas and pH data, $F.E.V._{1.0}$, R.C.M. and $\overline{P.A.P.}$ results in treated group patients at start of study.

Subject	Age (yr.)	Arterial PO_2 (mm Hg)	Arterial PCO_2 (mm Hg)	Arterial pH	$F.E.V_{1.0}$ (l)	R.C.M. (ml/kg)	$\overline{P.A.P.}$ (mm Hg)
14 M	65	48	57	7.38	0.50	41	36
15 M	63	58	57	7.39	0.70	44	33
16 F	67	47	63	7.38	0.51	48	23
17 M	59	53	50	7.36	0.61	35	33
18 M	45	60	59	7.39	0.55	38	27
19 F	55	57	57	7.35	0.55	44	30
20 M	63	52	57	7.39	0.95	37	31
21 F	67	47	52	7.39	0.20	25	39
22 M	66	48	62	7.33	0.35	48	52
23 F	59	57	51	7.39	0.65	26	33
24 M	63	59	49	7.41	0.95	38	24
25 F	55	59	55	7.40	0.50	40	46
26 F	59	55	57	7.34	0.70	57	35
Mean ± S.D.	60.5 ± 6.2	**53.8** ± 5.1	**55.8** ± 4.3	7.38 ± 0.02	0.58 ± 0.18	40.1 ± 9.2	34.0 ± 8.1

Table 2

Mean values ± 1 standard deviation for the control group patients, initially, and after 1, 2 and 3 years of the study, and for the patients receiving oxygen therapy, initially and after 1 year, 2 years and 3 years of the study. Significant differences between the two groups are indicated.

		Initial Assessment		1 Year Assessment		2 Year Assessment		3 Year Assessment	
		Control Patients	O_2 Therapy Patients	Control Patients	O_2 Therapy Patients	Control Patients	O_2 Therapy Patients	Control Patients	O_2 Therapy Patients
PaO_2 (mm Hg)	mean	**52.9**	**53.8**	57.0	57.0	53.0	46.6	50.0	46.0
	S.D.	4.8	5.1	10.4	6.4	11.8	7.6	1.1	10.0
	n	13	13	10	11	5	7	2	7
$PaCO_2$ (mm Hg)	mean	55.1	**55.8**	51.5	55.0	54.0	58.0	59.0	55.5
	S.D.	5.7	4.3	5.0	6.5	3.7	7.7	4.2	6.9
	n	13	13	10	11	5	7	2	7
Blood pH	mean	7.39	7.38	7.39	7.39	7.36	7.36	7.33	7.38
	S.D.	0.03	0.02	0.03	0.04	0.02	0.06	0.02	0.03
	n	13	13	10	11	5	7	2	7
Red cell pH	mean	7.17	7.17	7.13	7.13	7.14	7.12	7.05	7.13
	S.D.	0.05	0.03	0.09	0.11	0.06	0.09	0.02	0.04
	n	12	12	9	10	4	7	2	6
P.C.V. (ml/dl)	mean	50.8	54.0	48.0	46.5	52.0	45.0	57.0	44.0
	S.D.	3.9	7.3	7.2	8.5	5.2	4.8	1.0	5.3
	n	13	13	10	11	5	7	2	7
						← P<0.05 →		← P<0.02 →	
Hb (g/dl)	mean	15.6	16.1	15.1	14.1	16.0	13.6	17.4	13.1
	S.D.	1.1	1.8	2.4	2.0	1.9	1.4	0.4	1.6
	n	13	13	10	11	5	7	2	7
						← P<0.05 →		← P<0.01 →	
M.C.H.C. (g/dl)	mean	30.7	30.3	31.1	30.6	30.8	30.4	30.5	29.8
	S.D.	1.1	2.0	1.3	1.9	1.29	0.8	1.1	1.4
	n	13	13	10	11	5	7	2	7
$P_{50(7.4)}$ (mm Hg)	mean	27.3	26.8	27.3	27.9	27.2	26.8	30.1	26.3
	S.D.	1.7	1.0	1.3	1.3	1.5	1.2	0.8	2.1
	n	13	13	10	11	5	7	2	7
								← P 0.05 →	
2,3-DPG (μmole/gHb)	mean	16.53	15.53	14.86	14.50	15.27	13.43	17.00	14.78
	S.D.	2.59	1.74	1.82	2.58	4.32	2.21	3.30	3.47
	n	13	13	10	11	5	7	2	7
Hills 'n'	mean	2.68	2.67	2.86	2.91	3.00	2.84	2.72	2.84
	S.D.	0.31	0.15	0.32	0.32	0.34	0.27	0.09	0.48
	n	13	13	10	11	5	7	2	7

Table 3

Mean values ± 1 standard deviation for the 7 oxygen therapy patients who survived for 3 years of the study, and the 5 control group patients who survived for 2 years of the study. Significant changes within each group are indicated.

		Patients receiving oxygen therapy, n=7					Control patients, n=5		
		Initial Assessment	1 month Assessment	1 year Assessment	2 year Assessment	3 year Assessment	Initial Assessment	1 year Assessment	2 year Assessment
PaO_2 (mm Hg)	mean	54.3	51.7	54.7	46.6	46.0	55.0	59.8	53.0
	S.D.	5.31	7.0	3.6	7.6	10.0	2.2	9.1	11.8
		← P<0.02 →							
		← P<0.10 →							
$PaCO_2$ (mm Hg)	mean	56.0	54.3	55.1	58.0	56.0	54.0	52.4	54.0
	S.D.	4.08	7.4	5.0	7.7	6.9	2.6	4.2	3.7
Blood pH	mean	7.37	7.39	7.38	7.36	7.38	7.39	7.40	7.36
	S.D.	0.03	0.04	0.03	0.06	0.03	0.01	0.04	0.02
Red cell pH	mean	7.15	7.18	7.18	7.14	7.15	7.16	7.16	7.14
	S.D.	0.03	0.02	0.12	0.07	0.04	0.04	0.11	0.05
		(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=4)	(n=4)	(n=4)
P.C.V. (ml/dl)	mean	53.5	51.0	45.1	45.0	44.0	50.0	48.7	52.0
	S.D.	7.8	8.9	10.0	4.8	5.3	2.8	6.4	5.2
		← P<0.025 →							
		← P<0.02 →							
		← P<0.005 →							
Hb (g/dl)	mean	15.7	15.4	13.8	13.6	13.1	15.4	15.4	16.0
	S.D.	1.7	1.4	2.0	1.4	1.6	1.0	2.3	2.0
		← P<0.05 →							
		← P<0.02 →							
		← P<0.01 →							
M.C.H.C. (g/dl)	mean	29.5	30.4	30.9	30.4	29.8	31.1	31.5	30.8
	S.D.	1.2	2.6	2.1	0.8	1.4	0.4	0.7	1.3
$P_{50(7.4)}$ (mm Hg)	mean	26.8	28.0	27.8	26.8	26.3	27.0	26.5	27.2
	S.D.	1.2	2.1	1.4	1.2	2.1	1.9	0.5	1.5
		← P<0.02 →					← P<0.02 →		
2,3-DPG (μmole/gHb)	mean	15.91	13.96	13.60	13.43	14.78	15.53	14.78	15.27
	S.D.	1.75	3.76	3.24	2.21	3.47	1.25	1.30	4.32
		← P<0.05 →							
		← P<0.05 →							
Hills 'n'	mean	2.64	2.92	2.99	2.84	2.84	2.63	2.74	3.00
	S.D.	0.17	0.27	0.33	0.27	0.48	0.41	0.42	0.34

Table 4

Mean age, arterial blood gases and pH, and F.E.$V_{1.0}$ ($\pm$ 1 S.D.) in 47 patients with chronic bronchitis and emphysema.

Subjects	n	Age (years)	Arterial PO_2 (mm Hg)	Arterial PCO_2 (mm Hg)	Arterial pH	F.E.$V_{1.0}$ (l)
Smokers	30	59.2 $\pm$ 5.8	51.9 $\pm$ 5.4	53.1 $\pm$ 6.2	7.38 $\pm$ 0.04	0.60 $\pm$ 0.20
Non-smokers	17	62.7 $\pm$ 6.2	54.0 $\pm$ 3.9	50.1 $\pm$ 8.3	7.39 $\pm$ 0.04	0.60 $\pm$ 0.20
All patients	47	61.0 $\pm$ 6.1	53.0 $\pm$ 4.6	51.6 $\pm$ 6.9	7.39 $\pm$ 0.04	0.60 $\pm$ 0.20

Table 5

Arterial blood gas tensions, pH, oxygen and carboxyhaemoglobin saturations, red cell mass, and calulated renal venous oxygen tensions in all subjects.

Subjects	Mean COHb (%)	SaO_2 at 0% COHb (%)	SaO_2A at mean COHb level (%)	SaO_2T at mean COHb level (%)	PVO_2 assuming ($\Delta A-V$)=2ml/dl (mm Hg)	PVO_2 assuming ($\Delta A-V$)=5ml/dl (mm Hg)	R.C.M. ml/kg
SMOKERS							
1	4.5	78.0	78.0	74.5	37.5	30.4	37
2	3.0	86.5	86.3	83.7	41.4	31.0	32
3	8.0	79.5	79.9	73.5	35.4	27.1	48
4	9.0	85.0	85.6	77.9	40.5	29.6	48
5	4.2	83.5	85.3	81.7	38.1	30.0	35
6	14.2	80.5	89.7	77.0	30.8	26.6	47
7	6.9	86.0	83.3	77.6	36.8	28.7	44
8	10.5	79.5	82.5	73.8	36.0	28.0	30
9	5.4	90.0	88.5	83.7	37.5	32.4	41
10	12.0	86.0	85.5	75.2	37.0	25.6	57
11	4.5	82.5	81.3	77.6	36.5	27.6	52.5
12	10.0	90.0	91.6	82.4	45.2	34.5	55
13	6.6	89.5	94.0	87.8	42.5	30.3	42
14	3.5	82.5	81.3	78.5	38.6	30.5	41
15	8.0	79.5	79.5	73.1	33.7	23.7	51
16	8.0	88.5	87.7	80.7	41.7	31.2	44
17	6.1	85.5	85.9	80.7	40.6	31.1	35
18	6.6	90.5	92.4	86.3	43.1	31.2	38
19	4.6	80.5	81.3	77.6	36.7	28.6	43
20	5.1	85.8	82.5	78.3	41.9	32.7	43
21	12.5	86.5	97.3	85.1	42.2	34.7	35
22	9.0	90.0	94.6	86.1	41.9	31.6	36
23	7.1	90.0	93.0	86.4	42.1	33.6	32
24	6.8	89.0	97.2	90.6	43.6	33.5	33
25	5.1	89.5	87.0	82.6	44.8	33.4	36
26	10.0	84.0	84.5	76.1	39.0	29.4	41
27	11.0	65.0	74.0	65.9	28.2	22.2	59
28	3.3	83.5	82.0	79.3	41.0	32.7	43
29	12.0	91.0	99.0	88.0	44.8	35.0	38
30	8.9	86.5	87.6	79.8	39.7	29.2	54
Mean	7.5	84.8	86.6	80.1	39.3	30.2	42.4
S.D.	3.0	5.4	6.2	5.4	4.0	3.0	8.0
n	30	30	30	30	30	30	30
NON-SMOKERS							
31	2.5	86.0	88.5	86.3	39.8	29.3	29
32	2.8	82.0	78.5	76.3	40.0	30.3	28
33	4.2	86.5	86.5	82.9	42.9	34.8	29
34	3.3	85.0	79.5	76.9	47.4	37.0	28
35	1.4	90.0	89.0	87.8	43.5	31.7	23.5
36	2.2	85.0	85.0	83.1	39.4	30.2	25
37	2.4	90.0	93.0	90.8	39.6	30.9	27
38	2.1	85.0	85.0	83.2	37.8	28.1	26
39	3.8	82.5	83.0	80.0	41.4	31.8	31
40	3.0	90.0	90.0	87.3	44.5	34.1	32.5
41	1.1	88.0	84.5	83.6	44.9	34.0	31
42	0.7	90.0	90.0	89.4	41.5	31.9	25
43	3.5	91.0	89.5	86.4	49.5	39.1	31
44	2.8	88.0	88.0	85.5	44.6	35.7	42
45	4.2	83.0	83.0	79.5	38.6	30.9	31
46	3.0	92.0	89.0	86.3	51.0	39.0	29
47	3.2	86.0	83.5	80.8	44.6	35.6	29
Mean	2.7	87.0	86.2	83.9	43.0	33.2	29.2
S.D.	1.0	3.1	3.9	4.2	3.8	3.2	4.1
n	17	17	17	17	17	17	17

Table 6

Arterial blood gas tension, pH and $F.E.V_{1.0}$ determined on two occasions in those patients participating in the investigation of the effect of COHb on exercise tolerance (patients 1 - 9) and also the investigation of the C.D.H. effect in chronic bronchitis (patients 1 - 10).

Subject	Age (years)	Resting Arterial SO_2 (%)	Resting Arterial PO_2 (mm Hg)	Resting Arterial PCO_2 (mm Hg)	Resting pH	$F.E.V_{1.0}$ (l)
1 M	68	**87.2**	52.5	49.0	7.41	0.55
		90.2	53.5	47.0	7.41	0.35
2 F	61	**89.6**	54.0	46.5	7.41	0.40
		88.7	49.0	50.0	7.40	0.40
3 F	65	**88.4**	55.5	45.5	7.38	0.45
		89.7	56.0	46.5	7.40	0.50
4 M	58	**84.0**	48.0	51.0	7.40	0.65
		79.4	43.0	49.5	7.36	0.65
5 M	69	**90.8**	59.0	45.0	7.36	0.70
		89.4	56.0	43.5	7.37	0.70
6 M	60	**87.0**	47.0	43.0	7.39	0.55
		85.5	48.0	42.5	7.44	0.45
7 M	70	**85.6**	55.5	46.0	7.38	0.50
		86.9	51.5	43.0	7.42	0.60
8 F	54	**86.8**	56.0	42.0	7.34	1.90
		84.2	50.0	39.0	7.42	0.90
9 M	61	**84.6**	51.0	42.0	7.38	0.50
		85.6	50.0	42.0	7.43	0.50
Mean	62.9	**86.9**	52.0	45.2	7.39	0.63
S.D.	5.4	**2.8**	3.8	3.0	0.01	0.30
n=9						
10 M	66	**78.8**	46.0	43.5	7.37	0.40
		76.0	43.5	52.5	7.40	0.40
Mean	63.2	**85.9**	51.3	45.5	7.39	0.61
S.D.	5.2	**4.0**	4.2	3.1	0.01	0.29
n=10						

Table 7

The 12-minute walking distance, post exercise arterial O_2 saturation of total haemoglobin (SO_2T), and calculated femoral venous PO_2, assuming arterio-venous O_2 content differences of 7 and 10mls/100mls blood, on two separate occasions in nine patients with chronic bronchitis and emphysema, while breathing air and O_2, before and after in vivo elevation of carboxyhaemoglobin levels.

Subject	COHb (%)	POST-EXERCISE ARTERIAL O_2 SATURATION (SO_2T) (%)		CALCULATED FEMORAL VENOUS PO_2 ($\Delta(A-V)\ Co_2$ = 7mls/100ml) (mmHg)		CALCULATED FEMORAL VENOUS PO_2 ($\Delta(A-V)Co_2$ = 10mls/100ml) (mmHg)		12-MINUTE WALKING DISTANCE (METRES)	
		BREATHING AIR	BREATHING O_2	BREATHING AIR	BREATHING O_2	BREATHING AIR	BREATHING O_2	BREATHING AIR	BREATHING O_2
1	2.8	78	85	25.2	28.1	19.0	21.5	924	961
	13.0	-	-	-	-	-	-	789	819
	3.6	76	85	22.1	25.5	15.9	19.5	737	991
	14.5	78	84	19.1	21.4	12.2	14.7	672	922
2	1.8	77	92	24.4	30.8	18.1	23.7	804	740
	12.2	87	90	24.2	25.5	17.6	18.5	747	864
	2.9	81	94	24.6	30.4	19.1	24.0	829	877
	13.4	90	95	21.9	24.0	17.3	19.1	819	861
3	4.4	74	88	25.5	31.7	20.4	25.8	875	890
	13.8	-	-	-	-	-	-	778	888
	1.9	74	88	24.4	30.6	19.1	25.0	795	859
	11.7	66	88	16.9	20.3	9.6	19.6	721	819
4	2.6	-	-	-	-	-	-	919	910
	12.8	-	85	-	27.8	-	20.8	933	977
	4.0	80	88	27.8	31.3	22.9	26.0	944	963
	14.0	-	85	-	24.6	-	19.1	924	859
5	3.4	76	90	28.3	35.5	29.7	30.4	819	911
	11.8	71	80	23.9	28.2	19.0	23.0	839	903
	2.8	75	84	30.5	35.4	26.0	29.9	756	743
	10.5	81	87	29.9	33.6	24.1	27.0	672	756
6	1.6	80	86	24.9	27.3	18.5	20.9	776	720
	13.2	69	92	14.7	24.8	0	17.2	726	798
	2.9	83	93	24.8	33.1	14.9	20.7	672	756
	12.5	85	92	21.7	26.1	13.8	17.1	672	714
7	2.6	76	90	23.8	29.6	18.2	23.4	693	798
	12.3	79	86	17.7	21.2	11.7	14.1	756	789
	2.0	81	92	25.3	29.9	19.0	23.1	756	803
	11.0	85	86	21.8	21.8	14.6	14.7	693	756
8	2.9	82	90	28.3	32	22.6	25.8	882	777
	12.2	86	90	28.0	29.6	22.6	24.8	873	756
	2.6	65	85	20.8	27.9	13.8	21.6	962	898
	11.6	76	90	18.1	25.0	10.7	17.5	840	840
9	4.2	80	89	23.2	26.8	17.8	21.1	336	420
	13.7	72	91	14.1	23.0	7.5	15.8	252	336
	4.3	81	91	21.9	26.1	15.4	19.0	336	336
	11.8	77	90	15.5	22.2	0	14.0	168	222
MEAN	3.0	77.2	88.7	25.4	30.6	19.8	24.1	767.5	797.4
S.D.	0.9	4.5	3.2	2.5	2.8	4.1	3.2	177.9	174.2
MEAN	12.6	79.4	87.9	21.5	25.2	15.7	19.1	715.2	771.0
S.D.	1.0	7.7	3.9	4.5	3.6	4.8	3.8	201.5	191.9

Table 8

Mean $P_{50(7.4)}$, 2,3-DPG concentration, and plasma and red cell pH determined on two occasions in 9 patients with chronic bronchitis before and after in vivo elevation of COHb concentration.

		COHb<4.4%	COHb>10.5%
Mean $P_{50(7.4)}$ (mm Hg)		25.9	24.7
	1 S.D.	0.8	1.2
Mean 2,3-DPG (μmoles/gHb)	At rest	14.91	14.75
	1 S.D.	1.70	1.50
	Post exercise	14.26	14.96
	1 S.D.	1.90	1.80
Mean Plasma pH (venous)	At rest	7.30	7.32
	1 S.D.	0.04	0.04
	Post exercise	7.33	7.32
	1 S.D.	0.03	0.03
Mean red cell pH (venous)	At rest	7.05	7.06
	1 S.D.	0.08	0.07
	Post exercise	7.07	7.07
	1 S.D.	0.08	0.07

Table 9

Mean C.D.H. effect, 2,3-DPG concentration, $P50_{(7.4)}$, haemoglobin concentration and carboxyhaemoglobin saturation in normal men and women, and patients with chronic bronchitis and emphysema.

Subjects	C.D.H. Effect ($mM\ CO_2/mM\ O_2$)	2,3-DPG (μmole/g Hb)	$P50_{(7.4)}$ (mm Hg)	Hb Concⁿ. (g/dl)	COHb (%)
Normal Women					
Group A					
Mid menstrual cycle	0.34	15.04	28.0	13.5	2.1
S.D.	0.05	1.39	1.5	0.5	1.1
n	8	8	8	8	8
Menstruation	0.35	14.92	27.9	13.3	1.8
S.D.	0.07	0.96	1.3	0.4	1.0
n	8	8	8	8	8
Group B					
Mid menstrual cycle	0.34	15.11	26.8	13.8	1.7
S.D.	0.04	3.22	1.6	0.8	0.9
n	19	19	19	19	19
Menstruation	0.22	15.03	27.4	14.1	1.9
S.D.	0.04	1.82	1.8	0.9	1.0
n	19	19	19	19	19
Normal Men					
Mean	0.34	13.57	28.1	15.3	2.0
S.D.	0.03	1.10	1.8	1.2	0.8
n	7	7	7	7	7
Chronic Bronchitics					
Mean	0.30	14.90	25.8	15.5	3.2
S.D.	0.07	1.54	0.8	2.4	1.2
n	20	20	20	20	20
Mean	0.33	14.93	24.5	14.9	12.6
S.D.	0.08	1.47	1.3	2.3	1.1
n	20	20	20	20	20

Table 10

C.D.H. effect, reduced glutathione, ferritin, methaemoglobin, plasma haemoglobin and glycosylated haemoglobin levels in three normal women established on a course of oral contraceptives. (30μg Ethinyloestradiol, 0.15mg D-norgestrel) (Group B)

Subject	Phase of cycle	C.D.H. Effect ($mMCO_2/mM\Delta O_2$)	Reduced Glutathione (mg/l)	Ferritin (μg/l)	Met Hb (%)	Plasma Hb (g/l)	Hb A1c (%)
9	Mid	0.39	677.0	42	1.0	0.05	-
	Menstruation	0.29	-	32	-	-	-
14	Mid	0.30	1058.0	24	3.4	0.2	7.4
	Menstruation	0.22	1228.5	9	0.8	0.2	7.8
	Mid	0.29	1082.0	17	2.6	0.2	7.2
	Menstruation	0.25	1089.0	29	1.2	1.2	7.6
16	Mid	0.42	866.5	23	1.0	0.07	-
	Menstruation	0.23	795.0	133	1.8	0.01	-
	Mid	0.39	716.0	44	2.1	0.04	-
	Menstruation	0.23	1007.0	172	1.2	0.03	-

Table 11

CDH effect, haemoglobin concentration, 2,3- DPG concentration, $P_{50(7.4)}$ and carboxyhaemoglobin concentration in normal women immediately prior to beginning a course of oral contraceptives, and during the first two months of oral contraceptive treatment.

Subject	Phase in cycle	CDH Effect ($mM CO_2/mM\Delta O_2$)	Hb Conc^n. (g/dl)	COHb (%)	2,3-DPG (μmoles/gHb)	$P_{50(7.4)}$ (mmHg)	
17	Menstruation	0.46	13.2	2.0	15.00	26.1	← Start of Oral Contraception (30μg Ethinyloestradiol 0.15mg D-norgestrel)
	Mid	0.33	14.3	1.5	16.01	26.0	
	Menstruation	0.37	12.7	1.5	16.77	26.8	
	Mid	0.37	12.5	2.2	14.80	23.4	
	Menstruation	0.28	13.7	2.4	15.04	27.8	
18	Menstruation	0.37	13.0	1.8	15.15	27.7	← Start of Oral Contraception (30μg Ethinyloestradiol 0.15mg D-norgestrel)
	Mid	0.33	12.6	2.2	14.52	26.8	
	Menstruation	0.26	12.9	2.2	14.96	26.4	
	Mid	0.38	12.1	1.8	14.00	26.6	
	Menstruation	0.29	12.3	2.1	16.67	26.4	

Table 12

C.D.H. effect, reduced glutathione, ferritin, methaemoglobin, plasma haemoglobin and glycosylated haemoglobin levels in two normal women beginning a course of oral contraceptives. (Group C)

Subject	Phase of cycle	C.D.H. Effect ($mM CO_2/mM \Delta O_2$)	Reduced Glutathione (mg/l)	Ferritin ($\mu g/l$)	Met Hb (%)	Plasma Hb (g/l)	Hb A1c (%)	
17	Menstruation	0.37	1159.0	83	2.0	0.08	7.5	
								← Start of Oral Contraception ($30\mu g$ Ethinyloestradiol 0.15mg D-norgestrel)
	Mid	0.33	1070.0	13	1.0	0.08	7.8	
	Menstruation	0.26	1054.0	10	5.6	0.21	7.7	
	Mid	0.38	994.0	35	1.4	0.09	7.9	
	Menstruation	0.29	987.0	16	1.0	0.08	7.6	
18	Menstruation	0.46	902.0	134	1.0	0.14	-	
								← Start of Oral Contraception ($30\mu g$ Ethinyloestradiol 0.15mg D-norgestrel)
	Mid	0.33	1102.0	145	1.0	0.11	-	
	Menstruation	0.37	1146.0	89.	3.5	0.13	-	
	Mid	0.37	793.0	158	2.7	0.01	-	
	Menstruation	0.28	912.0	135	1.0	0.09	-	