# ON THE REACTION OF THE BLOOD IN THE BODY.

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CONTENTS.



During the course of some recent investigations on the causes of dyspnœa in patients in military hospitals[1] the desirability was felt of having further knowledge of the reaction of the blood as it exists in the body. It has been pointed out by Peters(1) that the present method of application of the hydrogen electrode to the determination of blood reaction inevitably leads to a more or less complete reduction of the blood, so that the results obtained apply only to blood which is carrying little or no oxygen. The reasons why the blood should become reduced during electrometric determinations are easily understood—in the first place it is exposed to an atmosphere of hydrogen under conditions which favour loss of oxygen; and secondly, as has been shown by Konikoff(2), a platinised platinum electrode is capable of catalysing the chemical combination between the hydrogen and the oxygen present as oxy-hæmoglobin. For this last-mentioned reason Michaelis recommends[2] that in dealing with oxygenated blood an excess of hydrogen should at first be taken in the electrode vessel in order to allow for that which will gradually combine with the oxygen. But apart from its action in reducing the blood, this combination of the hydrogen dissolved in the metallic platinum with the oxygen is liable to lead to considerable errors and uncertainties in the measurement of the E.M.F. since it leads to a

[1] See Lewis and others. *Brit. Med. Journ.* Oct. 14th. 1916.

[2] *Die Wasserstoffionen konzentration* (Berlin), p. 163. 1914.

depolarisation of the electrode: this means that the concentration of the dissolved hydrogen is not at its maximum saturation value, so that the E.M.F. set up is too small. In order to avoid these difficulties later workers on blood reaction have used only completely reduced blood (Peters(1)) or blood plasma (Milroy(3)).

When, in 1914, the work of Christiansen, Douglas and Haldane(4) on the $CO_2$ dissociation curve of blood appeared, it became evident that oxygenated blood absorbs less $CO_2$ at a given tension of that gas than does reduced blood at the same $CO_2$ tension. Recently Hasselbalch(5) has shown that the hydrogen-ion concentration of a dilute solution of sodium bicarbonate containing free carbonic acid may be calculated from the formula

$$p_H = p_{K_1} + \log \frac{[Bik]}{[CO_2]}$$

where $p_H$ is the exponent of the hydrogen-ion concentration according to Sorensen's notation, [Bik] represents the concentration of bicarbonate (combined $CO_2$) and $[CO_2]$ that of the free (dissolved) $CO_2$ in the solution, and $p_{K_1}$ is a magnitude which Hasselbalch defines as the $p_H$ when the concentrations of the free acid and of the salt are equal. This magnitude $p_{K_1}$ involves the value of the first dissociation constant of carbonic acid, and the degree of dissociation of sodium bicarbonate at various concentrations.

Assuming that this formula applies without modification to the free and combined $CO_2$ in blood, he has further pointed out that since the concentration of the combined $CO_2$ in oxygenated blood is less than that in reduced blood exposed to the same tension of $CO_2$, it is to be expected that oxygenation of blood in itself produces an increase in its hydrogen-ion concentration. It becomes essential then, in work on blood reaction, to have a method for the measurement of the $C_H$ not only of completely reduced, but more particularly also of oxygenated blood.

In attempting to analyse this effect of oxygenation on the reaction of blood it is most natural to suppose that when hæmoglobin takes up oxygen the oxyhæmoglobin produced is a relatively more acid substance, and that its formation inside the red corpuscle will be accompanied by a corresponding shift in the acid-base equilibrium in the corpuscle contents. But since the properties of the whole blood with regard to the absorption of $CO_2$ are also changed by the oxygenation it is evident that each shift in the acid-base equilibrium inside the corpuscle is accompanied by a corresponding change in the acid-base equilibrium of the

plasma. It does not follow that the reaction of the contents of the red corpuscle is the same as that of the surrounding plasma—in fact there is evidence that this is not the case (see the results and references given by Milroy (6) in his recent paper)—but the above considerations lead to the conception of a definite ionic equilibrium across the limiting membrane of the red corpuscle. In a recent paper de Boer (7) explains the change of distribution of $SO_4$ ion between corpuscles and plasma under varying conditions in terms of the same hypothesis.

Further, when one speaks of the reaction of the complex tissue blood, one strictly refers to the reaction of its plasma. It is this which is measured by an electrode immersed in the whole blood; the corpuscles merely remain suspended indifferently in the fluid, and may be removed without altering the E.M.F. reading. And doubtless it is the reaction of the blood plasma which comes into direct contact with the tissues that is of importance physiologically. Of course, towards a *change* of conditions—of $CO_2$ tension, for example—the separated plasma will behave differently from the whole blood because, as has already been explained, and will be evident from the results which follow, the corpuscles play a considerable part in determining the equilibrium condition. But when corpuscles and plasma are in equilibrium under any given conditions the removal of the corpuscles will produce no change in the ionic equilibrium provided that the conditions remain unchanged.

In this lies the principle which is fundamental to our method. The blood is brought into equilibrium with a gas mixture containing oxygen and $CO_2$ at any desired partial pressures: the corpuscles are then removed in such a way as to leave the equilibrium undisturbed, and the $p_H$ of the resulting plasma is measured electrometrically.

## I. *Methods.*

No attempt will be made to give a complete account of the electrometric method of measuring hydrogen-ion concentrations, since such already exists elsewhere (Sorensen (8)): all that is necessary is to describe those pieces of apparatus which have been found especially satisfactory for the purpose of the present experiments.

*The Saturator.* For the purpose of bringing a sample of blood rapidly into equilibrium with the mixtures of oxygen or hydrogen and $CO_2$, without the possibility of either partial laking or evaporation, a saturating vessel of the form and dimensions shown in Fig. 1 has been used. The central neck is for the introduction of the blood and is closed by a rubber stopper; the side tubes are for the passage of the gas-stream.

When in use the vessel is supported under each side tube so that it can be made to oscillate quickly about its points of support in order that the contained blood may be spread out as a thin film on the glass walls. It is important that the ends of the vessel should be of the shape shown in the figure so that the blood film is restricted to the central cylindrical body of the saturator; the side tubes then remain quite free from blood and no frothing occurs. The saturator is completely immersed in a thermostat bath at 37° C.: the gas mixture is first warmed by passing through a coil of narrow brass tube also immersed in the thermostat and is saturated with water vapour at 37° C. by bubbling through a little water in a small all-glass wash-bottle just before it comes into contact with the blood. Experiments to test the efficiency of this form of saturator have been made in two ways. Firstly, 3 c.c. samples of blood from the same specimen were exposed in it to a hydrogen-$CO_2$ mixture for varying lengths of time: it was found by a subsequent analysis of the blood that its $CO_2$ content became constant in 15 minutes. Secondly, when blood containing a large excess of $CO_2$ was used it was found that

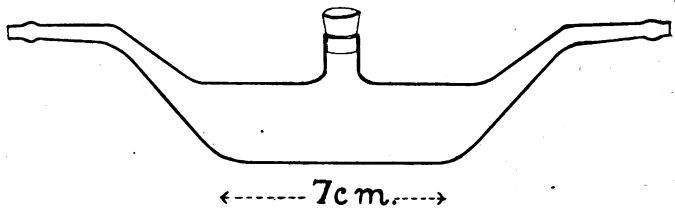


Fig. 1. The Saturator.

an exposure of the same length of time to the gas mixture was sufficient to reduce the $CO_2$ content to the same value as was found when blood containing little or no $CO_2$ was exposed to the same gas mixture. Also no laking of the red corpuscles occurs while the blood is in this saturator, for when the corpuscles are centrifuged off, the plasma is found to be quite free from any colouration due to free hæmoglobin. The particular piece of apparatus (centrifuge tube, electrode vessel, etc.) to which the blood is to be transferred after exposure to the gas mixture is usually connected to the exit tube of the saturator and immersed in the thermostat during the shaking, so that when equilibrium has been established it is necessary only to tilt the saturator and allow the blood to drain down into the side tube and then to be driven by the gas pressure into the adjoining piece of apparatus at a rate which is regulated by a clip on the connecting rubber tube. It is necessary to arrange the whole

apparatus so that it is completely immersed in the thermostat bath, with no exposed surfaces on which water vapour might condense. To ensure air-tightness all connections are made with rubber pressure tubing of capillary bore.

In order to obtain a sample of the gas for analysis, the gas mixture as it leaves the apparatus in the thermostat is led to a two-way tap, one exit of which opens to the atmosphere through a little water seal: the tap is in this position until the saturation of the blood is finished. Communication is then established with the burette of a Haldane's apparatus which has been previously filled with mercury. A convenient volume of the gas is drawn in, and then the two-way tap is again opened to the atmosphere in order to allow of the transference of the blood.

*The hydrogen electrode.* The electrode vessel that has been used for these experiments is similar to that described by Walpole(9) but is provided with a tap in its lower stem, and is of such size that it works conveniently with 1 c.c. of liquid. It is fitted with an air-tight rubber stopper through which pass *two* narrow glass tubes each carrying a separate electrode of very thin platinum foil. These electrodes reach almost to the bottom of the body of the electrode vessel so that their lower ends dip into the liquid even when there is only a small quantity of it present. By this means it is possible to use widely varying quantities of liquid for the determinations, according to the amount available. The object of using two separate electrodes in the one vessel is to make possible a confirmation of the correctness of the measurements by the agreement between the separate E.M.F. readings, any difference of reading being an indication that one, at least, of the electrodes is not functioning properly.

The usual experience with this apparatus is that at first the two electrodes give slightly different readings, but that when the electrode has been shaken for a few minutes both acquire the same value which then remains constant. Before each day's experiment the electrodes have been cleaned by thorough washing and heating to redness, and then replatinised, since the ease with which the platinum foil becomes saturated with hydrogen varies very considerably according to the condition of its surface.

As has been mentioned, the electrode vessel is connected in series with the saturator during the saturation so that the same hydrogen-$CO_2$ mixture passes through both: in this way not only the blood or plasma but at the same time also the platinum electrodes are brought into equilibrium with the gas mixture, so that when blood and electrodes are

brought into contact the E.M.F. between them is very rapidly set up. As soon as the blood has been transferred to it, the electrode vessel is disconnected from the saturator and immersed in a beaker of saturated potassium chloride solution kept at a temperature of 37° C. in a second thermostat. The calomel electrode is immersed in the thermostat alongside the beaker, and is so supported that its contact tube can be drawn up a little above the surface of the strong potassium chloride solution when a reading is not being taken. In order to detect any change of its E.M.F. due to diffusion of impurities into the calomel solution, the calomel electrode which is used in the experiments is frequently compared with a number of similar electrodes which are kept for use as standards only. As soon as one determination of the E.M.F. of the electrode system has been made, the hydrogen electrode is closed and shaken vigorously up and down for a few seconds by means of its glass support. A second reading is then taken and the shaking repeated until the readings given by the two platinum electrodes are identical and constant. It should be mentioned it has been found advisable, in order to avoid any error due to leakage of the current used for heating or stirring the thermostats, to disconnect these entirely from the mains at the moment a reading is being taken. With a similar object also, all the electrical measuring instruments are supported on large slabs of paraffin wax. From the following readings taken on a sample of plasma it will be seen that the E.M.F. increases slightly after the first one or two shakings, but acquires a constant value in not more than 15 mins.

| Time in minutes | 0 | 4 | 9 | 12 | 15 |
|---|---|---|---|---|---|
| E.M.F. observed (volts) | ·7805 | ·7818 | ·7827 | ·7827 | ·7823 |

As many of the experiments have been carried out at fairly high tensions of $CO_2$, the observed E.M.F. has in all cases been corrected for the reduction of the partial pressure of the hydrogen by the addition of ·17 millivolt for each 10 mm. of mercury partial pressure exerted by $CO_2$ (Sorensen(8), p. 417).

*The centrifuging apparatus.* In order to separate the corpuscles from a sample of blood without disturbing its equilibrium with a gas mixture it has been necessary to devise an arrangement for carrying out the centrifuging at body temperature. For this purpose the blood is transferred from the saturator, in the way already described, to a small centrifuge tube provided with a side tube and fitted up as shown in Fig. 2. This has a capacity of about 3 c.c. and is filled as completely as possible with the blood (a remaining bubble of gas mixture is of no

consequence). It is then closed off by means of well fitting glass rod stoppers and transferred to a cylindrical vacuum vessel which has been immersed in the thermostat bath and contains enough water at 37° C. to cover the centrifuge tube. The vacuum vessel is then at once transferred to the iron tube-holder of the centrifuge which, in order still further to diminish the loss of heat, has previously been warmed in boiling water. The whole arrangement is then placed in the centrifuge with as little delay as possible. With this arrangement the fall of temperature, which was noted in each experiment, of the water in the vacuum vessel was rarely more than 2° C. during the 10 minutes required for the centrifuging, and the change of temperature of the blood was probably less than this. At first some trouble was found in supporting the vacuum vessel so that it would not be broken during the centrifuging, but the arrangement shown in the figure has obviated the difficulty. The sealed off point of the vessel is protected by a cork, and it is supported on a cast of plaster of Paris which exactly fills up the space between the glass and the iron tube. The inner wall of the vessel is protected from pressure from the centrifuge tube by a large hemispherical pad of cork. When the centrifuging is finished, the plasma is at once pipetted off into a second saturator ready for the electrometric measurement.

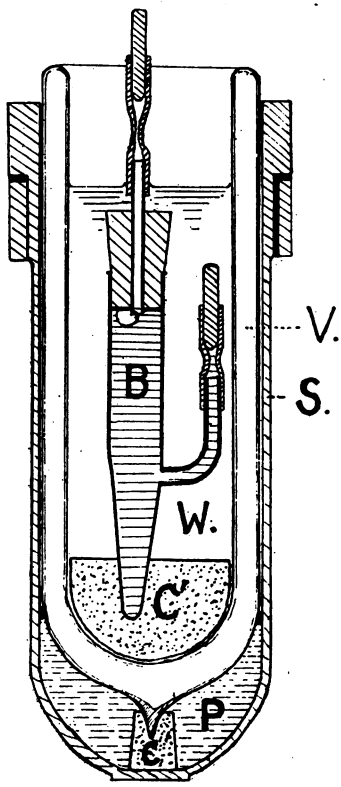


Fig. 2. Apparatus for centrifuging blood at body temperature. *B*, centrifuge tube completely filled with blood. *C'*, cork support for centrifuge tube. *V*, cylindrical vacuum vessel filled with water, *W*, at body temperature. It is supported by the plaster of Paris cast, *P*, and its sealed end is protected by cork, *C*. *S*, iron tube-holder of centrifuge.

The sample of blood for analysis was run off from the saturator into a pipette immediately after the centrifuge tube was disconnected, and transferred to the Barcroft differential apparatus. $\frac{N}{40}$ baryta was the

alkali used to retain the $CO_2$. In order to be certain of the absolute volumes of $CO_2$ liberated, the apparatus had been calibrated by an empirical method which was first suggested by Dr Itagaki and has not so far been described. Experiments were performed in which the $CO_2$ was liberated from 1 c.c. of sodium carbonate solutions of known strengths, under exactly the same conditions as those under which the $CO_2$ was liberated from the blood during an analysis. The readings obtained were plotted against the volumes of $CO_2$ calculated from the strengths of the carbonate solutions employed so that a curve was obtained from which the $CO_2$-volume corresponding to any given reading could be read off. This curve was very nearly, but not exactly, a straight line.

Lastly it may be mentioned that the mixtures of hydrogen or oxygen and $CO_2$ were stored in gas-holders over a saturated solution of calcium chloride in which $CO_2$ is but sparingly soluble.

For each pair of determinations about 10 c.c. of blood were obtained from my finger during the moderate activity occasioned by the preparation for the determinations. It was defibrinated by very careful whipping with a feather so that evaporation and damage to the red cells were reduced to a minimum. The sample was kept on ice until required.

With these methods and apparatus the order of experiment was usually as follows: In performing an 'oxygen' experiment about 4·5 c.c. of blood were brought into equilibrium with the oxygen mixture containing the required tension of $CO_2$. The exact value of this tension was determined by analysis, then 3 c.c. of the blood were centrifuged and the remainder used for the blood-gas analysis. The plasma was pipetted off into another saturator, and there brought into equilibrium with a hydrogen mixture containing the same tension of $CO_2$ as the oxygen mixture already used. This hydrogen-$CO_2$ mixture was also analysed and the plasma transferred to the electrode vessel and the E.M.F. reading taken at once.

The procedure for a 'hydrogen' experiment is identical with that for the 'oxygen' experiment, except that the blood, as well as the plasma after centrifuging, is treated with the hydrogen-$CO_2$ mixture, and so is obtained in a completely reduced instead of a fully oxygenated condition. Both 'oxygen' and 'hydrogen' experiments were always made on a portion of the same blood sample, and, with the exception of the first recorded experiment, were carried out consecutively on the same day. In some cases it was the 'oxygen,' in others the 'hydrogen'

experiment which was performed first, in order to avoid any possible error due to changes in the blood while standing on the ice for 3 or 4 hours. Actually it was found to be impossible to detect any difference in the results associated with the order in which the determinations were made.

TABLE I.

| No. | $CO_2$ tension (mm. Hg.) in oxygen mixture | $CO_2$ tension (mm. Hg.) in hydrogen mixture | $CO_2$ content of blood (c.c. per 100 c.c.) oxygenated | $CO_2$ content of blood (c.c. per 100 c.c.) reduced | E.M.F. (volts) against N/10 calomel electrode (fully corrected) plasma from oxygenated blood | E.M.F. (volts) against N/10 calomel electrode (fully corrected) plasma from reduced blood | $C_H$ plasma from oxygenated blood $10^{-7}\times$ | $C_H$ plasma from reduced blood $10^{-7}\times$ | $p_H$ plasma from oxygenated blood | $p_H$ plasma from reduced blood | Change of $p_H$ due to oxygenation at constant $CO_2$ tension |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 36·9 | 37·4 | 46·0 | 49·9 | ·7853 | ·7909 | ·479 N | ·389 N | 7·32 | 7·41 | −·09 |
| 2 | 2·3 | ·79 | 10·9 | 12·5 | ·8563 | ·8584 | ·034 | ·031 | 8·47 | 8·51 | −·04 |
| 3 | 19·1 | 19·6 | 36·7 | 41·2 | ·8014 | ·8045 | ·263 | ·234 | 7·58 | 7·63 | −·05 |
| 4 | 70·0 | 72·1 | 64·7 | 69·2 | ·7785 | ·7805 | ·617 | ·569 | 7·21 | 7·25 | −·04 |
| 5 | 10·2 | 10·1 | 30·7 | 32·2 | ·8118 | ·8164 | ·178 | ·148 | 7·75 | 7·83 | −·08 |
| 6 | 5·7 | 8·1 | 17·7 | 28·0 | ... | ·8190 | ... | ·135 | ... | 7·87 | ... |
| 7 | 54·8 | 55·3 | ... | 58·9 | ·7833 | ·7840 | ·513 | ·490 | 7·29 | 7·31 | −·02 |
| 8 | 33·9 | 33·4 | 43·8 | 49·9 | ·7928 | ·7959 ? | ·363 | ·317 ? | 7·44 | 7·50 ? | −·06 ? |
| 9 | 6·0 | 5·7 | 21·7 | 24·2 | ·8220 | ·8240 | ·120 | ·110 | 7·92 | 7·96 | −·04 |

## II. *Experimental Results.*

It has thus been possible at each partial pressure of $CO_2$ to determine the $CO_2$ content and also the hydrogen-ion concentration of both the fully oxygenated and the completely reduced blood. The results are summarised in Table I.

TABLE II. Direct measurements on reduced blood.

| $CO_2$ tension (mm./Hg.) | E.M.F. (volts) against N/10 calomel electrode (fully corrected) | $C_H$ $10^{-7}\times$ | $p_H$ |
|---|---|---|---|
| ·6 | ·8473 | ·048 N | 8·32 |
| 11·8 | ·8127 | ·170 | 7·77 |
| 22·9 | ·8007 | ·269 | 7·57 |
| 38·8 | ·7914 | ·380 | 7·42 |
| 49·4 | ·7872 | ·447 | 7·35 |
| 70·0 | ·7803 | ·575 | 7·24 |

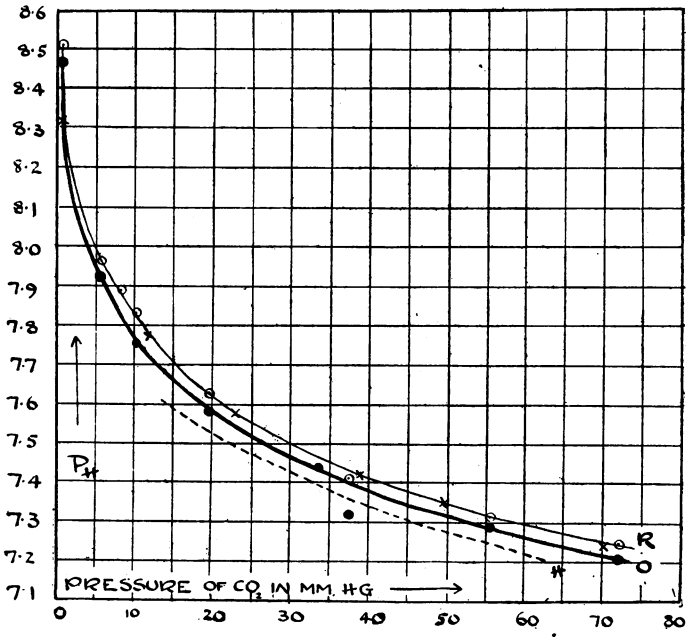


Fig. 3. Curve *R*, completely reduced blood. Curve *O*, fully oxygenated blood. *X*, direct measurements on reduced blood. *H*, Hasselbalch's curve.

It might have been expected that the behaviour of the blood towards $CO_2$ would have been much more variable than the relation of its hæmoglobin to oxygen, for in the first case the relations appear to be much more complicated than in the latter. But in spite of the fact that all

these determinations had to be made on separate samples of blood collected at different times during the course of a month, the values in Table I when plotted yield very satisfactory curves. Fig. 3 shows the relation between $p_H$ and $CO_2$-tension for both the fully oxygenated and the completely reduced blood. The two curves are parallel to one another, at a distance representing $\cdot 038\, p_H$, so that at any given $CO_2$ tension, a change from the condition of complete reduction to full oxygenation is accompanied by a decrease of ·038 in the $p_H$. Also in Fig. 3 are plotted some values, shown in Table II, obtained by a somewhat different method for my blood in a completely reduced condition. In these experiments, which were performed some months before those recorded in Table I, a sample of 2 c.c. of blood was exposed in a saturator to an analysed mixture of hydrogen and $CO_2$ and then passed direct into the electrode vessel, no centrifuging being necessary. Thus the E.M.F. reading was taken with the *whole* reduced blood in contact with the electrode. It will be seen that the points obtained in this way lie on exactly the same curve as is obtained by the present method in which the blood is centrifuged, and only the plasma used in the electrode vessel. The exact correspondence between the results obtained by these two methods can leave no doubt as to their validity, and as to the correctness of the contention that, *under equilibrium conditions*, the reaction of blood as measured electrometrically is identical with the reaction of its plasma.

In Fig. 3 also I have copied, as faithfully as the scale of his figure permits, the latest curve given by Hasselbalch (*loc. cit.* p. 126) showing the change of $p_H$ with $CO_2$ tension in his own blood at 38° C. Hasselbalch uses a mixture of air and $CO_2$ in his saturator, and transfers the fully oxygenated blood to his electrode. It thus seems fairer to draw a comparison between his curve and the one which represents the behaviour of oxygenated blood. It will be seen that the general direction of the curves is the same, but that Hasselbalch's blood appears to be more acid than mine at each $CO_2$ pressure. The reason for this may lie to a certain extent in an individual variation, and without more data of this kind for the bloods of a number of individuals the extent to which this factor operates must remain undecided. But the divergence may possibly be partly explained by differences in our experimental procedure. It is a significant fact that practically all the errors which are likely to occur in electrometric determinations on blood (with the exception of loss of $CO_2$, which is out of the question in the experiments here described) tend to produce a reduction in the value of the E.M.F.

with a consequent shift of the result towards the acid side. The particular point in which Hasselbalch's procedure differs from our own is that he runs oxygenated blood into the electrode vessel, and so his results are liable to be effected by an error due to depolarisation of the electrode. This, as mentioned on p. 440 causes the E.M.F. reading to be too low, *i.e.* the blood to appear more acid.

As these experiments furnished a series of values of the total $CO_2$ content of both oxygenated and reduced blood under various known tensions of $CO_2$, it was thought to be of interest to calculate from them the $p_H$ of my blood at various $CO_2$ tensions according to Hasselbalch's method (*loc. cit.*). The $CO_2$-dissociation curves were first plotted (they are not here reproduced) and from them corresponding values of $CO_2$ tension and total $CO_2$ content were read off and substituted in Hasselbalch's formula. The results are shown in Table III. Without plotting

TABLE III. Calculation of the $p_H$ of blood by Hasselbalch's method from its total $CO_2$ content.

| $CO_2$ tension (mm. Hg.) | Total $CO_2$ content (c.c./100 c.c.) of blood | | $p_H$ of blood | |
|---|---|---|---|---|
| | oxygenated | reduced | oxygenated | reduced |
| 20 | 37 | 41 | 7·52 | 7·56 |
| 40 | 48 | 52 | 7·30 | 7·34 |
| 50 | 53·2 | 57 | 7·24 | 7·27 |
| 60 | 58·5 | 62·5 | 7·20 | 7·23 |

them in the form of curves it will be seen that the calculated variations in $p_H$ with $CO_2$ tension run quite parallel with those observed experimentally, and the mean difference between the $p_H$ of the oxygenated and the reduced blood under the same $CO_2$ tension is $-$ ·035, which is in excellent agreement with the value obtained from the experimental curves (Fig. 3). The values of the $p_H$'s are, however, consistently lower than those found by experiment.

It has been stated above that the difference of $p_H$ between the oxygenated and reduced blood at any $CO_2$ tension appears to remain constant at $-$ ·038, whatever the actual value of the $CO_2$ tension may be. This is expressed by the parallelism of the curves in Fig. 3. It should be borne in mind, however, that this interval in $p_H$ corresponds to a larger interval in actual concentration of hydrogen ions ($C_H$) at the lower values of $p_H$ than at the higher ones so that there is a greater difference in $C_H$ of the bloods at higher $CO_2$ tensions than at low ones. (See curves *O* and *R* in Fig. 7.)

Further—by plotting the relationship between the $p_H$ of the blood,

and its total $CO_2$ content (Fig. 4) it will be seen that the change of reaction which occurs on oxygenation in a sample of blood whose total $CO_2$ content is unchanged is much greater than if it were kept at constant $CO_2$ tension. At a $CO_2$-content of 50 c.c. $CO_2$ per 100 c.c. blood—a concentration which occurs under ordinary physiological conditions—the difference in $p_H$ amounts to as much as $-\cdot 11$.

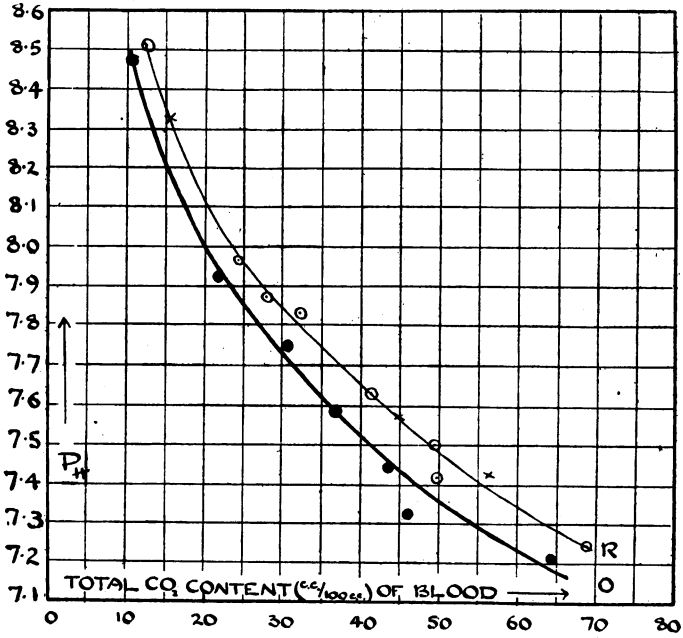


Fig. 4. Curve *R*, completely reduced blood. Curve *O*, fully oxygenated blood. *X*, direct measurements on reduced blood.

### III. *The variations in the reaction of the blood in the body.*

These last-mentioned curves showing the change in $p_H$ of blood as the $CO_2$-content increases are of importance in another way—for with their aid it is now possible to represent the actual reaction changes of the blood as they occur in the body. For convenience those portions of the curves which occur within the physiological limits are drawn on a larger scale in Fig. 5. The argument is precisely similar to that used by Christiansen, Douglas and Haldane in dealing with the changes of $CO_2$ tension in the body: we shall assume with them that the tension of $CO_2$ in the alveolar air is 40 mm. Hg.: that the arterial blood is practically completely saturated with oxygen: that if all the oxygen

were used up in the production of $CO_2$ a completely reduced blood containing an excess of 15 c.c. $CO_2$ per 100 c.c. would result, but, that actually during its passage round the circulation the blood loses only about one-third of its total oxygen.

From the results in Table I it follows that at a tension of 40 mm. Hg. of $CO_2$ my blood when completely oxygenated will contain 48 c.c. $CO_2$ %. Its $p_H$ will then be 7·38 (point *A*) (Fig. 5). If no change in $p_H$ occurred as a consequence of loss of oxygen, the addition of 15 c.c. $CO_2$ % would cause a change of $p_H$ to 7·20 (point *B*). But owing to the simultaneous

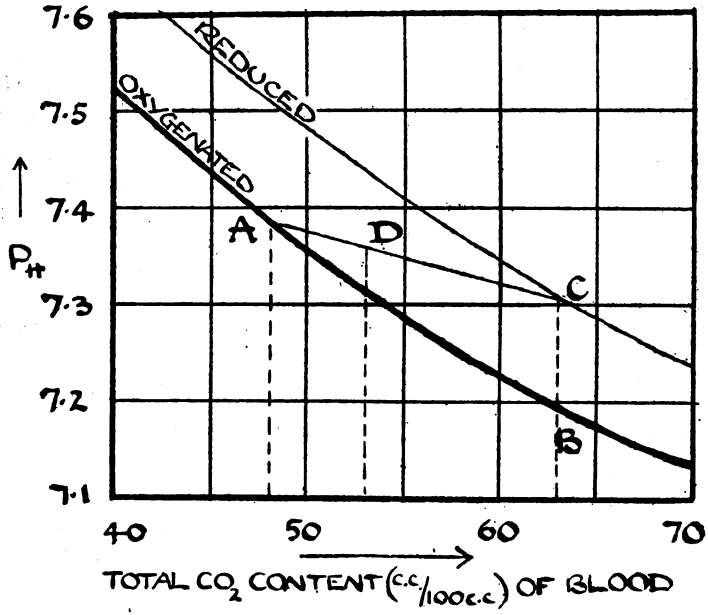


Fig. 5.

effect of loss of oxygen on the reaction the $p_H$ falls only to 7·31 (point *C*) —a change of ·07 instead of ·18. Thus the change of reaction of the blood as it loses oxygen and gains, at the same time, an equivalent amount of $CO_2$ is represented by the straight line *AC*, and this must be a close approximation to the conditions obtaining in the body. Actually, since in the body the blood probably never becomes more than one-third reduced, the change of reaction will be represented by some such portion of the line as *AD*, and the total change of $p_H$ during a circulation will then be about ·02. This is in exact agreement with Hasselbalch's estimate (5), p. 131).

It should be emphasised that in all these considerations we have been dealing with the properties of blood which has been defibrinated, and is, in that respect, abnormal as far as the body is concerned. In this connection the remarks of Christiansen, Douglas and Haldane ((4), p. 248) on the possible effects of defibrination are particularly significant. Moreover, the representation of the changes occurring in the body by the straight line *AD* involve the assumptions that the loss of oxygen by the blood and the acquisition of an equivalent quantity of $CO_2$ occur simultaneously; that the amount of change of reaction of blood due to loss of oxygen alone is directly proportional to the degree of reduction which occurs: and that $CO_2$ is the only product of oxidation. Doubtless these statements are approximately true, but we have no data at present which enable us to decide exactly what form the line *AD* should take.

In comparing this account of the variations in the reaction of blood with that of Christiansen, Douglas and Haldane of the variations in its $CO_2$-tension one cannot but be convinced that, of the two alternative explanations which they put forward ((4), p. 258) the former is the more probable—that oxygenation assists in the expulsion of $CO_2$ from the blood, not on account of any change in the state of aggregation of the hæmoglobin molecules, but by virtue of the increased hydrogen-ion concentration to which it gives rise. The fact that the difference in $p_H$ between oxygenated and reduced blood as calculated from the $CO_2$-dissociation curves by Hasselbalch agrees with that observed experimentally is further evidence in support of this explanation of the effect of oxygenation.

In a later paper it is proposed to attempt to analyse the various factors which determine the extent of the change of reaction of the blood in the body as represented by the line *AD*. For the present, attention will be called only to the fact that one of the circumstances which are of importance in determining the length of the line *AD* (*i.e.* the total difference of reaction between the venous and the arterial bloods) is the distance between the curves for oxygenated and for reduced blood respectively. Now this distance is a measure of the effect of oxygenation in changing the reaction of the blood, and is, therefore, entirely determined by the red corpuscles. Evidently then the total variation of reaction of the blood in the body is dependent not only upon the composition of the plasma, but also upon the presence of the red corpuscles. Had we further information it might be possible to decide exactly what is the relative importance of these two factors: all that can be said now is that they appear to exert influences which are of

the same order of magnitude on the ionic equilibrium in the blood. Nor should this result be surprising when one remembers what a relatively large bulk of the whole blood is made up by the corpuscles.

This conclusion is of importance in two ways. In the first place it now becomes evident that this effect of the red corpuscles in determining the blood reaction must exert some influence in cases where there exists a considerable alteration in the number of red corpuscles present in the blood. The change of reaction on oxygenation will, presumably, be at least roughly proportional to the number of the red corpuscles present.

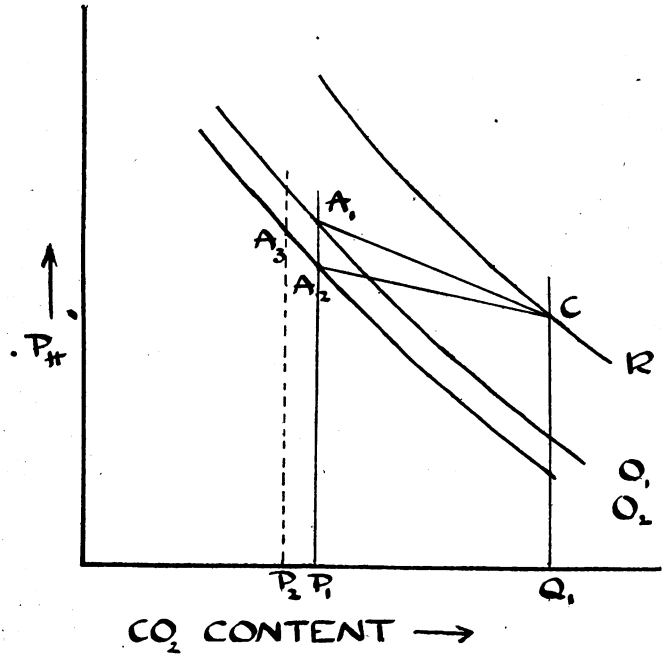


Fig. 6. See description in text.

Now, in Fig. 6, let the curve $R$ represent the change of $p_H$ with increased $CO_2$-content in a completely reduced blood, and $O_1$ the same relation for the fully oxygenated blood (1) containing a normal concentration of red corpuscles $n_1$. If from some cause the concentration of red cells rises to $n_2$, the behaviour of the plasma *alone* remaining the same as before, we may represent the properties of the more concentrated blood, (2) by the curve $O_2$ where

$$\frac{\text{distance between } R \text{ and } O_1}{\text{distance between } R \text{ and } O_2} = \frac{n_1}{n_2}.$$

Let $P_1Q_1$ represent the volume of $CO_2$ which would be produced in excess of the normal content, $P_1$, of the arterial blood at the normal alveolar $CO_2$ tension, if all the oxygen were converted into $CO_2$.

If we neglect any possible effect on the reaction or $CO_2$-dissociation of the completely reduced blood due to the presence of the excess of *reduced* red corpuscles, the condition of both bloods will be represented by the point $C$. But when the blood loses $CO_2$ and gains oxygen in the lungs that containing the concentration of red cells $n_1$ will change to the condition $A_1$, but that containing $n_2$ corpuscles will remain abnormally acid as indicated by the point $A_2$. Of course, in the body the venous blood $n_2$ will also be more acid than the venous blood $n_1$, but owing to the partial reduction, the difference will not be so great as between the arterial bloods, and, on the assumption that no effect is produced on the reaction of the completely reduced blood by the excess of red corpuscles, the difference would become nil if all the oxygen in the blood were converted to $CO_2$. The abnormally acid arterial blood $n_2$ will stimulate the respiratory centre so that a condition of hyperpnœa will be set up. The alveolar $CO_2$-tension will fall to a value corresponding to a $CO_2$-content in the oxygenated blood represented by $P_2$, for example, at which respiratory equilibrium is attained with the $p_H$ of the arterial blood at a value represented by $A_3$—still abnormally acid.

It now remains to give some idea of the possible magnitude of the effects which can arise in this way from an altered concentration of red corpuscles. According to Campbell, Douglas and Hobson(10) a rise of 2 mm. in the alveolar $CO_2$ pressure causes an increase of 10 litres per minute in the total ventilation of the lungs. From Fig. 7 it will be seen that for my blood at 40 mm. $CO_2$ tension a rise of 2 mm. means an increase of about $\cdot 014 \times 10^{-7}$ in the hydrogen-ion concentration. The total change in $C_H$ caused by the change from complete oxygen saturation to complete reduction is approximately $\cdot 043 \times 10^{-7}$ at a constant tension of 40 mm. $CO_2$. The change due to oxygenation of venous blood two-thirds saturated will therefore be $\cdot 014 \times 10^{-7}$. Now supposing that the number of red corpuscles in my blood has a normal value of 5 millions per mm.$^3$, and that by some means this number were to become increased to 7 millions—an extent of increase very commonly observed in pathological conditions. The change on oxygenation of the venous blood will now be

$$\frac{\cdot 014 \times 7}{5} \times 10^{-7}, \textit{ i.e.} \text{ nearly } \cdot 020 \times 10^{-7}.$$

The effect, then, of an increase of the red corpuscles from 5 to 7

millions per mm.$^3$ on the reaction of the fully oxygenated arterial blood (represented by $A_1A_2$ in Fig. 6) is an increase of $(\cdot020 - \cdot014) \times 10^{-7}$, *i.e.* of ·006 in the $C_H$. This is about 43 % of the total rise in $C_H$ required to increase the total lung ventilation by 10 litres per minute. A change of concentration of red corpuscles may, therefore, constitute a very appreciable factor in the production of respiratory disturbances.

Secondly, it follows that, since the corpuscles have so large a share in determining the ionic equilibrium in the blood, the changes of reaction

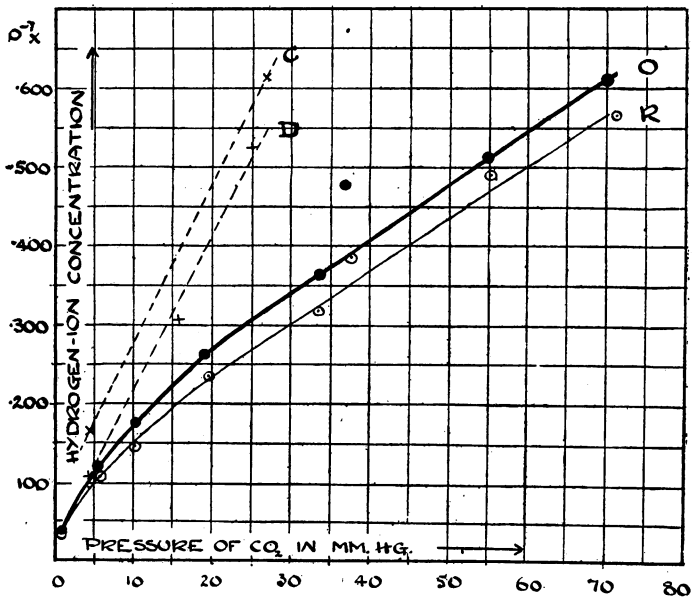


Fig. 7. Curve *R*, completely reduced blood. Curve *O*, fully oxygenated blood. Curve *C*, Milroy's curve for plasma from Cat. Curve *D*, Milroy's curve for plasma from Dog.

observed in the plasma when the $CO_2$ tension to which it is exposed varies do not furnish a reliable indication of the changes which would occur in the *whole* blood as a result of an equal change in $CO_2$ tension. To illustrate this I have copied in Fig. 7 the curves given by Milroy (6) showing the change in $C_H$ in plasma with increasing tensions of $CO_2$ alongside the corresponding curves for my own whole blood. Unfortunately Milroy publishes only the values he obtained with plasma from the cat and dog, but they serve to illustrate the enormous difference of behaviour which results from the absence of the blood cells.

## IV. *Note on the change of reaction produced in blood by carbon-monoxide.*

During the preliminary experiments for this research the hydrogen used for the electrometric determinations was obtained from a cylinder. It was discovered subsequently the gas contained carbon-monoxide in sufficient quantity to saturate the hæmoglobin of the blood samples exposed to it to the extent of about 95 %. This means that in these experiments the reaction of fully oxygenated blood was being compared, not with that of reduced blood, but with that of blood practically completely saturated with carbon-monoxide. The results are recorded in Table IV: they are all probably a little low—but each to the same

TABLE IV. Carbon-monoxide experiments.

| $CO_2$ tension in hydrogen mixture (mm. Hg.) | E.M.F. (volts) against N/10 calomel electrode* plasma from oxygenated blood | E.M.F. (volts) against N/10 calomel electrode* plasma from CO-blood | $p_H$ plasma from oxygenated blood | $p_H$ plasma from CO-blood |
|---|---|---|---|---|
| 36·6 | ·7899 | ... | 7·40 | ... |
| 18·6 | ·7993 | ·8011 | 7·55 | 7·57 |
| 46·1 | ·7828 | ·7832 | 7·28 | 7·29 |
| 71·6 | ·7781 | ·7774 | 7·20 | 7·19 |
| 1·0 | ·8451 | ·8459 | 8·29 | 8·30 |
| 9·9 | ·8078 | ·8082 | 7·69 | 7·70 |
| 28·4 | ·7925 | ·7937 | 7·44 | 7·46 |

* Not corrected for the partial pressure of CO present in the hydrogen.

extent—as no correction has been applied for the reduction of the partial pressure of the hydrogen by the carbon-monoxide present. It is interesting to find that the carbon-monoxide has the same effect in increasing the hydrogen-ion concentration of the blood as the oxygen, just as it also tends to expel the $CO_2$. This is another piece of evidence in support of the view that oxygen reduces the absorbing power of blood for $CO_2$ by virtue of the increase of acidity which it produces.

## SUMMARY.

1. Under equilibrium conditions, the hydrogen-ion concentration of blood as measured electrometrically is the same as that of its plasma.

2. The $p_H$ of fully oxygenated blood at a given tension of $CO_2$ is less than that of completely reduced blood at the same $CO_2$ tension by ·038: this difference is constant for all tensions of $CO_2$ and its value agrees with that calculated from the $CO_2$-dissociation curves by Hasselbalch's method.

3. The difference in $p_H$ between arterial and venous blood in the body is approximately ·02.

4. The red corpuscles play a large part in determining the ionic equilibrium in blood: the changes in reaction produced in blood plasma by increase of $CO_2$ tension are much greater than those produced in the whole blood.

5. An increase of the relative number of red corpuscles to the extent observed in pathological conditions exerts an appreciable effect on the reaction of the blood in the body, and so on the respiratory equilibrium.

6. The effect of oxygen in expelling $CO_2$ from blood is due to the increased acidity it produces.

7. Carbon-monoxide has the same effect as oxygen in increasing the acidity of blood.

I am indebted to my wife for sharing with me the experimental work involved in this investigation. Without her help it would have been impossible to carry out the simultaneous determinations which were required. My best thanks are due to Mr Barcroft under whose direction I have worked for much helpful advice, and to Prof. Milroy for an opportunity of working in his laboratory. But especially am I indebted to Capt. Peters, M.C., R.A.M.C. This indebtedness is due for much of the apparatus which was set up by him in the Cambridge Laboratory and for the use of it. The work which forms the subject of this paper is roughly that in which Capt. Peters was engaged. I have verified his statements that his former curve(11) erred on the side of acidity and that over the physiological portion it is differentially correct; and I find in his note-book, that on August 1, 1914, he obtained readings for CO-blood which agree with mine. Mr S. D. Sturton, of Emmanuel College, has helped with some of the blood gas analyses.

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