



THE HALDANE-SMITH METHOD OF ESTIMATING  
THE OXYGEN TENSION OF THE ARTERIAL  
BLOOD. By W. A. OSBORNE.

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## 1. General theory.

THE method of estimating the oxygen tension of arterial blood which was introduced by Haldane and Smith<sup>1</sup> is based upon the fact that haemoglobin forms a dissociable compound not only with oxygen but also with carbon monoxide, and that when haemoglobin in solution is exposed to a mixture of carbon monoxide and oxygen for an interval of time sufficient for the establishment of equilibrium, the ratio of the pressure of the carbon monoxide to the pressure of the oxygen in the air (and of course in the solution) is a constant multiple of the ratio of the concentration of the carboxyhaemoglobin to the concentration of the oxyhaemoglobin. We may express this relationship as follows:

$$\frac{\text{pressure of carbon monoxide}}{\text{pressure of oxygen}} = K \frac{\text{concentration of carboxyhaemoglobin}}{\text{concentration of oxyhaemoglobin}}$$

or simply

If now the amount of haemoglobin which is unattached either to oxygen or CO be negligibly small, as it is in the experiments in question, we may write equation (1) as follows :

<sup>1</sup> *This Journal*, xx. p. 497. 1896.

<sup>2</sup> Bohr from theoretical grounds would expect this equation to read

$$\frac{p_{CO}}{p_{O_2}} = K \left( \frac{C_{COHb}}{C_{O_2Hb}} \right)^2,$$

but the experiments of Haldane and Smith and of Hüfner do not bear this out. (See article by Bohr in Nagel's *Hdb. d. Physiol.* I. p. 78, 1905.)

where  $a$  is the percentage saturation of the haemoglobin with CO. If then  $a$ ,  $K$ , and  $p_{CO}$  are known we can at once determine the value of  $p_{O_2}$ . In the experiments of Haldane and Smith an animal breathed a definite percentage of carbon monoxide mixed with air; when equilibrium was supposed to be established, the percentage saturation of the haemoglobin of the animal with CO was determined by a colorimetric method. It was invariably found that this percentage saturation with CO was lower than that obtained by subjecting blood *in vitro* to the same mixture of gases. If these experiments were correctly performed and if no physical explanation of the fact is forthcoming, we are compelled to adopt one of the following assumptions:

1. The lung does work by keeping the carbon monoxide from entering.
2. The lung does work by forcing the oxygen inwards.
3. The lung does work both by keeping the carbon monoxide out and by forcing oxygen in.

As regards these three alternatives we may at once follow Haldane and Smith and pronounce nos. 1 and 3 untenable on account of the rapid absorption of CO by the lung and the fact that this gas is not a protoplasmic poison, at least with the metazoa.

The pressure of the CO in the air breathed, subject to a slight reduction owing to the admixture with water-vapour in the alveoli, will therefore give us  $p_{CO}$ . The constant  $K$  can be determined from the curve of dissociation of carboxyhaemoglobin in the presence of a constant pressure of oxygen. A simple calculation will then give a value for  $p_{O_2}$ , or the oxygen tension in arterial blood. Using a colorimetric method (to be criticised later) for determining  $a$  and  $K$ , Haldane and Smith, as a result of their experiments, announced that the oxygen tension of arterial blood was higher than that of the air breathed, in the case of the human being as much as 38.5 % of an atmosphere<sup>1</sup>. If this conclusion is correct the absorption of oxygen by the lung is not a purely physical process; on the contrary the lung is a secreting organ doing work by forcing oxygen into the blood against pressure.

Abandoning the teleological argument against this view, namely that increased oxygen tension means only a trivial increase in the amount of oxygen absorbed, as also the argument from analogy, namely that wherever work is done in the body we generally find cells rich in protoplasm, I shall confine myself to a criticism of those portions of the

<sup>1</sup> This *Journal*, xxii. p. 237. 1897.

investigation of Haldane and Smith which are based upon experimental evidence.

## 2. *The determination of K.*

By means of his colorimetric method Haldane in 1895<sup>1</sup> obtained a curve of dissociation of carboxyhaemoglobin in the presence of air, that is, in the presence of oxygen at a pressure of 20.9 % of an atmosphere. This curve is partly reproduced in curve (1) in Fig. 1. In 1896 Haldane and Smith<sup>2</sup> using the same method but excluding the disturbing influence of strong sunlight altered this curve and gave it the form reproduced in curve (2). In 1897<sup>3</sup> the same investigators, using the same method, but allowing a longer time for the establishment of equilibrium, obtained a curve (3) which is

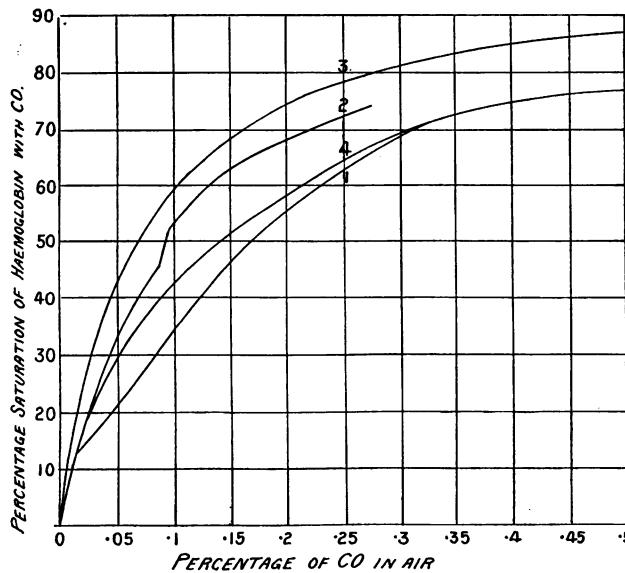


Fig. 1.

a rectangular hyperbola and from which for the first time a value could be given to the constant  $K$ , namely 0.0032. It might be thought that finality had been reached when so regular and definite a curve had been obtained and that no further investigation on the subject was called for. But this is by no means the case, for

<sup>1</sup> This *Journal*, xviii. p. 451. 1895.

<sup>2</sup> This *Journal*, xx. p. 506. 1895.

<sup>3</sup> This *Journal*, xxii. p. 233. 1897.

Hüfner<sup>1</sup>, by means of his spectrophotometer, obtained a curve (4) which is likewise a rectangular hyperbola and from which the constant  $K$  can be calculated as 0'0064 or exactly double that obtained from Haldane and Smith's curve. It is obvious that either the method or conditions of experiment must be wrong in one case or the other or in both. Haldane, moreover, in 1895<sup>2</sup> obtained by this same colorimetric method a curve for the dissociation of carboxyhaemoglobin in the absence of oxygen. This curve is again a rectangular hyperbola, but a reference to Haldane and Smith's work in 1897 shows that this curve has been completely repudiated. The values found in these latter experiments are also considerably higher than those obtained by Bock<sup>3</sup> with the absorptiometer, a fact which Bohr is inclined to attribute to the different concentrations of haemoglobin employed.

When one considers the fact that the whole question as to the curve of dissociation of oxyhaemoglobin is at present in a very unsettled condition<sup>4</sup> no surprise need be felt, when the much more difficult determinations of the values for carboxyhaemoglobin are found to give discrepant results.

### 3. The colorimetric method.

This colorimetric method for determining the relative concentrations of carboxyhaemoglobin in presence of oxyhaemoglobin is well known and need not be described in detail. In principle the method consists in adding to a definite amount ( $a$  c.cs.) of dilute blood, a volume of standard carmine solution ( $b$  c.cs.) so that the tint is identical with the tint of the blood to be examined which has been equally diluted. Then a further amount of carmine ( $c$  c.cs.) is added until the tint is equal to blood equally diluted but saturated with carbon monoxide. The percentage saturation ( $x$ ) of the blood examined will be obtained from the simple proportion

$$\frac{b+c}{a+b+c} : \frac{b}{a+b} :: 100 : x,$$

that is to say the ratio between the concentration of the carboxyhaemoglobin present and the maximum concentration possible, equals the ratio between the concentrations of carmine. The principle of the method is, as far as I am able to judge, sound, but in the technique there are undoubtedly errors. At the very outset it may be observed that two

<sup>1</sup> *Arch. f. exp. Path. u. Pharm.* XLVIII. p. 99. 1902.

<sup>2</sup> *loc. cit.*

<sup>3</sup> *Centralbl. Physiol.* VIII. p. 385. 1894.

<sup>4</sup> See for instance Loewy, *Archiv. f. Anat. u. Physiol. (Physiol. Abt.)*, 1904, p. 231.

fundamental laws of colorimetry have been violated. First the solutions to be compared are contained in tubes of circular section, the light passing through the tubes perpendicular to their length. The field of colour is in each case not uniform and, as the tubes act as cylindrical lenses, some of the peripheral rays do not reach the eye. In the second place the tubes are some distance apart whereas the two fields of colour ought to be separated by a theoretical partition. These two errors must of necessity prevent one from obtaining consistent results and such has been my personal experience, though I have followed the directions of Haldane and Smith implicitly and have further made a series of experiments with different concentrations of blood and of carmine. I have prescribed the method to students of science and medicine who had shown considerable skill in titrimetric and other analyses but they could never obtain by it a consistent series of observations. I do not doubt that prolonged acquaintance with the method will reduce the range of discrepancies but, speaking from my own experience, I have been unable, though I have worked with the utmost care, to obtain results which could be regarded as having any scientific value. Even in the protocols of experiments which Haldane and Smith publish at the close of their last paper the absence of a sharp end-point is noticeable. Thus in the first experiment<sup>1</sup> we find

"A. Titration up to tint of animal's blood,

2.7 c.cs. of carmine not quite enough	
2.9 c.cs. right	}
3.1 c.cs. , ,	
3.3 c.cs. , ,	
3.5 c.cs. slightly too much.	

B. Titration up to tint of same blood saturated with CO,

5.4 c.cs. not quite enough	
5.7 c.cs. right	}
6.0 c.cs. , ,	
6.3 c.cs. , ,	
6.6 c.cs. slightly too much."	

It will be seen that three values are given for each end-point, and if we take in series each one of the three values in the first determination in conjunction with each one of the three values in the second determination we get values for the percentage saturation varying between 65.9 and 74.6.

<sup>1</sup> *loc. cit.* 1897, p. 255.

Apart from the inconsistencies due to working with cylindrical lenses placed some distances apart, there is another source of error which I had detected and had spent some time in confirming, before I discovered that it was admitted by Haldane and Smith themselves. This is the fact that, with alterations in the quality of the light, the amounts of carmine used in the two titrations do not alter *pari passu*, and in consequence different values may be assigned to the percentage saturation of a blood solution of constant quality. Haldane and Smith state<sup>1</sup> "...reference to the notes shows the apparent difference to have been brought about by slight changes in the quantity of carmine necessary to reproduce the tint of complete saturation. In many cases the light was failing and at the same time changing in quality towards the end of the experiment, and when this was the case less carmine was needed to reproduce the saturation tint." It has always been my experience that the titrations performed towards evening varied in a marked manner from those done earlier on the same day or on the following morning; usually I obtained a higher percentage. Not only does this irregular variation occur with evening light but I have been able to detect it by day when the background against which the tubes were held was in any way varied. Working with the same solutions of blood and carmine I have noticed within half an hour that the amounts of carmine added were altered according as I held the tubes against blue sky, grey cloud, whitewashed wall, or frosted window. These alterations were greater than could be accounted for by the ill-defined end-point given by the tubes and invariably consisted not only in an alteration in the absolute but also in the relative amounts of the carmine added in the two titrations. Haldane, in his article on the Detection and Estimation of Carbonic Oxide in Air<sup>2</sup>, utters an explicit warning that the titration should not be carried out "in extra bright light, or in dull weather, or towards evening," but I have been able to observe changes in a "fairly well-lighted room in bright weather" which altered the determinations very considerably. I have even noticed a change when a red brick building opposite one of the windows of the room, but not that at which I was working, was strongly illuminated with sunlight. A special series of experiments made with blood solution 50% saturated with CO, failed to discover to what apparent alteration in light these changes were due. Generally I got a high reading towards evening, but on some evenings I got the very

<sup>1</sup> loc. cit. 1896, p. 513.

<sup>2</sup> This Journal, xx. p. 521. 1896.

reverse. Nor were my efforts successful in using as a background a screen in a well-blinded room illuminated by a projection lantern either with white or coloured light, as the end-points, particularly the full saturation end-point, were very indistinct.

My experience with the method points therefore to the conclusion that the readings obtained by it cannot be taken as even approximately correct, unless at the same time a control determination is carried out with a blood solution containing a known percentage of carbon monoxide which should be as near as possible to that of the solution to be examined. Even with this improvement the method employed could not give exact determinations for the two reasons already specified.

#### 4. *The absorption of carbon monoxide.*

One and a half hours were taken by Haldane and Smith as a sufficient time for equilibrium to be established between the carbon monoxide breathed and that contained in the blood of the body. That is to say in one and a half hours all, or at least by far the greater part, of the blood of the body must pass through the lungs and remain there a sufficient time for equilibrium to be established. That there should be for one and a half hours a "rapid absorption" of carbon monoxide and that then this rapid absorption should "suddenly cease" is not what we should expect from the general principles of the circulation. Attempts to displace the entire blood of an animal by saline solution teach one how stagnant the blood flow is in various veins. That similarly sequestered blood in the normal animal eventually reaches the lungs no one doubts, but its arrival must be spread over a considerable interval of time. Moreover it is generally recognised that complete equilibrium between gas and fluid, as in determinations of solubility of a gas in a liquid, takes a very long time to come about and is practically never attained in ordinary experiments. One would expect therefore that the rate of CO absorption would remain at a maximum for the minimum total circulation time and would then fall in a gradual manner but not actually ceasing until after a very long interval.

That absorption of CO had not been complete and that therefore the tension of CO must have been lower in the blood than in the alveolar air constitutes the main objection of Schmid<sup>1</sup> to Haldane and Smith's conclusions. Without doubt complete equilibrium between the blood and the alveolar CO had not been attained, but in answer to this it may

<sup>1</sup> Schmid, *Pflüger's Arch.* cxiii. 612. 1906.

be urged that if Haldane and Smith were unable to detect any increase during the two hours following the first one and a half hours they were justified in assuming that the equilibrium was sufficiently well approximated for their purpose and in ruling this consideration out as a possible source of fallacy.

#### 5. *The permeability of the lung to carbon monoxide.*

It is evident that the degree of permeability of the lung to CO will influence the time taken for equilibrium to be established and not the final tension of the gas in the blood. The question of permeability is therefore resolved into the question, just discussed, of the absorption of the carbon monoxide.

#### 6. *The effects of carbonic acid.*

As Haldane and Smith discovered, and as one might expect, the tension of carbon dioxide in the venous blood cannot alter the distribution of haemoglobin between the oxygen and carbon monoxide at the tension in which these exist. I have carried out an experiment in which 0.5% blood was exposed to 0.058% CO in air and to 0.059% CO in a mixture containing CO<sub>2</sub> 9.7%, O<sub>2</sub> 21.3%, the remainder being nitrogen. No difference in the tint could be detected.

#### 7. *Oxidation or other consumption of carbon monoxide in the body.*

That carbon monoxide is not oxidised in the body may be taken as proved by the researches of Gaglio<sup>1</sup>, Haldane and Smith, and Wachholz<sup>2</sup>, whilst the same researches dispose of the idea that CO is bound or used up by the tissues, a view put forward by Schmid<sup>3</sup> but supported by no evidence except the presence of after-effects of CO poisoning.

#### 8. *Leakage of CO from the blood.*

It is evident that if CO is present in the blood it must eventually pervade every cell in the body and penetrate to the centre of the non-vascular tissues, such as cartilage, etc.; moreover it must pass into the urine and alimentary secretions and, in experiments with man, leave the body by the skin. This source of error has not been investigated by Haldane and Smith, but it is obvious that the leakage of CO from the

<sup>1</sup> *Archiv. f. exp. Pathol.* xxii. 236. 1887.

<sup>2</sup> From abstract in *Biochem. Centralbl.* v. 132. 1906.

<sup>3</sup> *loc. cit.*

blood, though present, must be very small. The tension of the CO in the blood is low, barely half a millimetre of mercury, whilst the amount held by the haemoglobin is relatively large. In the tissues where the blood becomes venous, the haemoglobin, by its loss of oxygen, must take up some of the CO dissolved in the plasma, thus producing an appreciable fall in the tension of this gas. Loss by the skin was absent in those experiments where the animal was enclosed by the CO-containing air, whilst, in the case of man, a comparison with the CO<sub>2</sub> output, where the tension is at least one hundred times greater, shows that the skin-loss must be very trifling.

#### 9. *The influence of the counter-diffusion of oxygen and carbon dioxide.*

In 1870 Wretschko<sup>1</sup> at the instigation of Loschmidt conducted an investigation on the diffusion of two gases in presence of a third gas. A cylindrical vessel was divided into two equal cylindrical parts by a partition which could be withdrawn, thus allowing complete communication between the two halves, without, however, allowing any communication with the outside air. Each cylindrical half was filled separately with the gas mixture, the apparatus was then placed vertically so that the heavier mixture was below, the shelf was withdrawn, and diffusion allowed to take place for a definite interval of time. The partition was then readjusted and analyses made of the constituents of each half, after sufficient time had been given for the composition of the mixture in each half to become uniform. It was invariably found that, if two gases were allowed to diffuse into each other in the presence of a third gas which, *before* the diffusion, was present in the same percentage in each half of the cylinder, *during* diffusion the third gas was present to a greater extent in the upper than in the lower half. For instance, a mixture of 75% CO<sub>2</sub> and 25% H<sub>2</sub> was allowed to diffuse into a mixture of 75% O<sub>2</sub> and 25% H<sub>2</sub>. At the end of 75 minutes the upper half contained 25.3% CO<sub>2</sub> and 25.31% H<sub>2</sub>, whilst the lower half contained 24.27% H<sub>2</sub>. These experiments of Wretschko at once suggest a possible source of error in Haldane and Smith's researches. In the lung there is a constant outward drift of CO<sub>2</sub> and a constant inward drift of O<sub>2</sub>, the volume of each gas being practically the same. If then it could be proved that the outward diffusing CO<sub>2</sub> hindered the absorption of CO more than the inward drift of O<sub>2</sub> helped the absorption of CO, we

<sup>1</sup> *Sitzungsb. d. k. Akad. d. Wissensch. Wien*, LXII. 575. 1870.

should have a purely physical reason why the CO of the blood can never attain to the tension of that gas in the alveolar air. It is true that, in the lung, diffusion takes place through a membrane which was not represented in Wretschko's experiments. It would be difficult to say whether in the substance of the membrane the third gas would be equally swept along by the heavier gas in its diffusion. But at the alveolar surface of the lung-membrane there is that inflowing current of oxygen and outgoing current of carbonic acid which would be analogous to the free diffusion described. As, in Wretschko's experiments, the diffusion occurred in a closed system it is obvious that the amounts of O<sub>2</sub> and CO<sub>2</sub>, which diffused downwards and upwards in unit time, were almost identical.

I have made a series of experiments on the same plan as that employed by Wretschko with the object of testing whether when CO<sub>2</sub> is allowed to diffuse into O<sub>2</sub> in the presence of a small amount of CO there is the same heaping up of the latter gas.

In the first place it will be necessary to discuss the methods of gas analysis employed. The apparatus used was the improved Haldane form<sup>1</sup> in which a pipette for the combustion of gases by a hot platinum spiral is present, in addition to the potash and pyrogallic pipettes. Haldane has drawn attention to possible sources of error which I have also had frequent occasion to observe, such as leakage at the rubber junctions and presence of sulphides in the potash. These errors are however easily prevented by a little care in the setting up of the apparatus and its subsequent manipulation. But there are other sources of error which Haldane has not mentioned and which I will here give in detail.

1. I have found that the first analysis of a given sample of air may differ very markedly from the second as regards CO<sub>2</sub> content, whilst the second, third, and following analyses are consistent. This error can be obviated by driving the residual gas in the burette into the potash bulb before the analysis, and then bringing the potash to its mark. I am not quite clear as to the cause of this error or of its prevention; possibly the wetting of the glass in the potash levelling bulb makes some difference either by surface tension or simply by lowering the level through subtracting a small quantity of fluid from the column. The mixing together of the contents of the potash absorbing bulb and its levelling bulb may also partly explain the difference.

<sup>1</sup> *The Investigation of Mine Air*, by Sir C. Foster and J. S. Haldane, London. 1905.

2. On no account should the potash be drawn to any considerable height above its mark, otherwise a layer of viscid potash solution clings to the inside of the tube and therefore adds to the apparent volume of the gas. Neglect of this precaution may produce serious errors when small percentages of a gas ( $\text{CO}_2$  or  $\text{CO}$ ) are estimated.

3. I have been quite unable when working with percentages of  $\text{CO}$  under  $0.1\%$  to draw conclusions of any value from the diminution in volume which follows combustion in the appropriate pipette. In the first place the mercury in this pipette is necessarily far less sensitive than the potash column, and accuracy in bringing it to its mark harder to obtain. More important still is the fact that the air in the connections between the combustion pipette and the burette is decidedly warmer than the air in the latter receptacle. Driving the burette air backwards and forwards from the burette into the combustion pipette has little value, for, on connecting the burette with the potash pipette, the mercury in the combustion pipette may be seen to rise gradually from cooling of the air between it and the upper three-way tap. If a long time be allowed to elapse in order that this connection-air may come to the temperature of the room it is generally found that the control gauge has altered so much that a proper correction is out of the question, for, as Haldane admits, the control is not quite accurate and changes of any considerable magnitude cannot be compensated. It is also possible that there may be some hysteresis of the rubber tube passing from the combustion pipette to its levelling tube. This tube must dilate under the pressure necessary to expose the platinum wires, and its return may not be a complete one or may take a considerable time. Fortunately, in the experiments to be mentioned, the only combustible gas present was carbon monoxide so that I could discard the diminution and depend solely on the amount of  $\text{CO}_2$  formed on combustion.

4. In a number of control experiments I found that, on leading fresh pure air into the combustion pipette and heating the wires by the electric current, carbon dioxide or other gas absorbable in potash was formed in appreciable amounts. This I at first attributed to the fact that the lubricant in the tap had been softened by the heat and had crept down the tube and been burned. This source of error was easily removed, but again I detected a formation of  $\text{CO}_2$  or other combustion product. This was eventually traced to the mercury which contained combustible material, probably grease. On cleaning out the pipette and levelling tube and filling again with mercury which had been bubbled some days under strong  $\text{H}_2\text{SO}_4$  I at last obtained a series of negative

results with air and consistent positive results with a mixture of CO and air. After a time, to my no small annoyance, the error appeared again. I could think of no other reason for this than that the rubber stopper and the rubber tube had given off sulphur or other combustible material which either alone or combined with the mercury had been burned. It was in fact noticed that, on depressing the mercury, the platinum wires did not emerge clean, for, on turning on the current, a dull glow was obtained which rapidly brightened whilst vapours of some description could be seen eddying in the air-space around. If the rubber stopper and rubber tube were done away with and glass connection only employed, the levelling, which is infrequently used, could be brought about by adding or removing mercury with a fine pipette, but, unfortunately, I do not see how such a connection could exist and freedom to change or repair the platinum coil retained. In the experiments described I frequently replenished the mercury, and before every analysis tested the apparatus with pure air. Yet taking all the precautions mentioned and relying solely on the amount of CO<sub>2</sub> formed on combustion, I was never able to get exactly consistent results in estimations of CO in air when the amount of this gas was present to the extent it would be in experiments conducted on animals, namely about 0·06 %. In consequence I carried out most of the experiments to be described with mixtures containing larger percentages of CO.

Two metal cylinders *A* and *B* (Fig. 2), each with an internal diameter of 7·2 cms., were joined in such a manner that a thin metal plate could be thrust between the two, completely separating one cavity from the other, or could be partly withdrawn, allowing complete communication between the cylinders but preventing access to the outside air. Beeswax mixed with vaseline was used as a lubricant. Each cylinder had two outlet tubes which could easily be stoppered or connected with the gas analysis apparatus. The cylinder *A* had a content of 1100 c.cs., *B* of 1090 c.cs. The gas mixtures were made in glass gas-holders, the oxygen mixture being contained over distilled water, the carbonic acid mixture over water saturated with that gas. A day was allowed to elapse before the mixtures were led into the respective cylinders and an analysis made immediately before doing so. Into the cylinder *A*, previously filled with water saturated with CO<sub>2</sub>, was led carbonic acid with no detectable

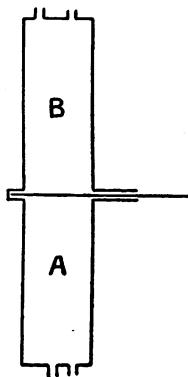


Fig. 2.

impurity except the CO added. Into *B* the oxygen mixture was led. The oxygen on analysis (pyrogallic absorption) proved to be 96 % which may be taken as pure as the remainder consisted of nitrogen which differs but little in molecular weight from oxygen. The cylinder *B* was filled at first with water half-saturated with CO<sub>2</sub>, but, in the last three experiments, with distilled water which could make no appreciable error by absorption, for I found on unscrewing the apparatus that the amount of water which clung to the wall was negligibly small. In neither case was the gas, on being led in, allowed to bubble through the water, but by various obvious devices was allowed simply to displace the water and a current of gas mixture maintained for some time through the cylinder after the water had been driven out. When each cylinder was filled its outlet tube was immediately stoppered. The apparatus was then placed in a vertical position in a sheltered corner of the laboratory with the cylinder *A* containing the CO<sub>2</sub> mixture below. The shelf was then withdrawn and after an interval of 55 minutes was once more pushed in between the cylinders. One of the outlet tubes of each cylinder was then connected with the gas analysis apparatus; a sample was sucked out and driven backwards and forwards for a time (in order to mix the contents of the cylinder) and finally retained and analysed. As each sample contained considerable quantities of CO<sub>2</sub>, loss of which in the potash bulb would bring the mercury into the uncalibrated part of the burette, the mixture, after being driven into the potash bulb, was imprisoned there for a short time and a sufficient quantity of air admitted into the burette which was then mixed with the gas over the potash and the mixture completely freed from CO<sub>2</sub>. Analysis of the CO was then made in the usual way, the calculation being based on the fresh CO<sub>2</sub> formed rather than on the diminution in bulk on combustion. The table on p. 61 gives the results of the experiments made<sup>1</sup>.

Though these values found will not allow any quantitative conclusions to be drawn, they prove fairly convincingly that small quantities of CO can be heaped up in the direction of the diffusing carbon dioxide. That this takes place in the lung can hardly be doubted though to what extent this action is reduced by the presence of a membrane, by dilution with nitrogen and by the wide extent of the area of diffusion it would be difficult to say. But one is justified in stating that this action of the counter diffusion of oxygen and carbon dioxide is a possible source of

<sup>1</sup> I have omitted two experiments where an attempt was made to use small percentages (0.05) of CO, but the values given for *A* and *B* before diffusion were so divergent that the final result would possess no interest.

error which Haldane and Smith have not dealt with and which must vitiate their conclusions.

*Results of analysis of carbon monoxide.*

In percentages.

Experiment	Before diffusion		After diffusion		Temperature in degrees centigrade
	A	B	A	B	
I	0.26	0.26	0.23	0.28	11—11.5
II	0.11	0.11	0.07	0.13	12—13
III	0.26	0.26	0.26	0.28	11—12
IV	0.27	0.28	0.25	0.28	13—14.5
V	0.25	0.25	0.23	0.27	13
VI	0.09	0.08	0.06	0.09	14—14.5
VII	0.14	0.13	0.13	0.14	12—12.5
VIII	0.27	0.28	0.28	0.33	11—12
IX	0.07	0.05	0.06	0.07	13

CONCLUSIONS.

1. Haldane and Smith were not justified in concluding from their researches that the oxygen tension of arterial blood is higher than that of the alveolar air for
  - a. The colorimetric method employed cannot give sufficiently accurate estimations of carboxyhaemoglobin;
  - b. No account was taken of the counter-diffusion of oxygen and carbon dioxide on the carbon monoxide.
2. Some possible sources of error in using Haldane's gas analysis apparatus are described.

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