SOME EXPERIMENTS ON THE SOLUBILITY OF GASES IN OX BLOOD AND OX SERUM

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(*Received October 10th, 1910*)

The question of the solubility or absorption of gases, more especially oxygen and carbon dioxide, in blood is one which has claimed the attention of many physiologists.¹ Until a few years ago the purely chemical explanation of absorption was practically undisputed; the absorption of oxygen by blood being attributed chiefly to the formation of a compound with the haemoglobin, and of carbon dioxide to the reputed alkalinity of blood and the formation of carbonates and bicarbonates. Recently, however, the remarkable surface actions of colloidal substances have been increasingly investigated by physical chemists, and it was suggested by Findlay and Harby,² and by Wo. Ostwald,³ that the absorption was due, for the most part, to the surface action of the colloids in blood. This view was fully discussed by Wo. Ostwald⁴ on the basis of the experimental material then available.

As scarcely anything was then known regarding the influence of colloids on the solubility of gases, we investigated the influence of a considerable number of colloids and fine suspensions on the solubility in water of carbon dioxide and nitrous oxide⁵ under a range of pressures from 760 mm. to about 1,400 mm. of mercury. The results obtained were much more complex than the simple adsorption theory led us to expect; but one of the main conclusions reached was that surface adsorption is not sufficient in itself to produce a marked increase in the solubility of a gas in water at atmospheric pressure. On the contrary,

1. See, for example, Bohr's monograph *Blutgase und respiratorischer Gaswechsel* in Nagel's *Handbuch der Physiologie des Menschen*.

2. *Kolloid Zeitschr.*, III, 169, 1908.

3. *Ibid.*, II, 264, 1908.

4. *Loc. cit.*

5. *Trans. Chem. Soc.*, XCVII, 536, 1910.

in the few cases in which an increased solubility was observed, it seemed most suitable to attribute the effect, except in the case of charcoal and silicic acid, to chemical combination.

In order to compare the behaviour of blood and blood-serum with the behaviour found in the case of the colloids previously investigated, we carried out a few determinations of the solubility of oxygen, carbon dioxide, carbon monoxide, nitrous oxide, and nitrogen in defibrinated ox blood and ox serum, under pressures varying from that of the atmosphere up to about 1,400 mm. of mercury.

The method employed by most physiologists for determining the amount of gas absorbed by blood or serum has been to pump off the dissolved gas and to measure the volume of gas so pumped off. We have adopted the opposite course, and have measured the volume of gas *taken up* by the liquid under different pressures.¹ Since the solubility of a gas is expressed by the ratio concentration of gas in liquid to concentration of gas in the gas space, it follows that since these two factors increase together with increase of pressure for gases obeying Henry's law, the solubility-pressure curve will in such cases be a straight line parallel to the pressure axis. It differs, therefore, from the curve of absorption, which is a continuously ascending curve. This point requires to be emphasised, since we have employed the solubility co-efficients, whereas biologists use, as a rule, the absorption co-efficients.

The temperature at which the solubility was determined was, in all cases, 25·0°, and as absorbing liquids we used 'natural' ox blood and ox serum as obtained from the slaughter-house, as well as 'deaerated' blood and serum, i.e., blood or serum which had been kept under a reduced pressure of about 15 mm. of mercury for an hour.

I. SOLUBILITY OF CARBON DIOXIDE

For the purpose of these experiments commercial carbon dioxide was employed, containing 0·58 per cent. of impurities. The solubility in pure water was found to be 0·817, the solubility being, in accordance with Henry's law, independent of the pressure. The solubility in blood and serum is given in Table I, the results being also exhibited graphically in fig. 1.

1. For a description of the apparatus employed and the formula for calculating the solubility, see *Geffcken, Zeitschr. physikal. Chem.*, XLIX, 298, 1904; Findlay and Creighton, *Trans. Chem. Soc.*, XCVII, 537, 1910.

FIG. 1.—PRESSURE IN MM. OF MERCURY

TABLE I

				‘ Natural ’ Ox Blood. Density 1.067				
Pressure in mm.	...		762	882	985	1,050	1,115	1,291
Solubility of CO ₂	...		1.276	1.236	1.201	1.181	1.162	1.149
				Deaërated Ox blood. Density 1.067				
Pressure	...	...	758	850	937	1,061	1,232	1,301
Solubility	...	...	1.461	1.401	1.362	1.309	1.245	1.226
				Natural Ox serum. Density 1.025				
Pressure	...	...	761	879	989	1,050	1,179	1,331
Solubility	...	...	0.956	0.935	0.919	0.910	0.906	0.907

In all these cases both the blood and the serum quickly became saturated with the gas, and even after ten or twelve hours no further quantity of gas was taken up.

II. SOLUBILITY OF OXYGEN

Gas obtained by heating pure potassium permanganate; passed over soda lime. The solubility in pure water (mean of five series) was found to be 0.0294. Geffchen¹ found 0.0308; Bohr and Bock,² 0.0317; Winkler,³ 0.03097.

TABLE II. Solubility of Oxygen in Blood and Serum

				Natural ox blood.		Density 1.064		
Pressure in mm.	...	...	756	918	1,061	1,243	1,342	1,481
Solubility	...	...	0.1180	0.1054	0.0957	0.0861	0.0832	0.0779
				Natural ox serum.		Density 1.027		
Pressure in mm.	...	...	752	871	1,003	1,192	1,346	1,523
Solubility	...	...	0.0016	0.0028	0.0042	0.0067	0.0081	0.0091
				Deaërated ox serum.		Density 1.027		
Pressure in mm.	...	...	746	901	1,187	1,256	1,390	1,551
Solubility	...	...	0.0056	0.0081	0.0123	0.0135	0.0162	0.0183

See also Figs. 2 and 3. In the figures E-blood and E-serum represent deaërated blood and serum.

The point of saturation was quickly reached, and no further absorption occurred after a period of twelve hours.

1. *Zeitschr. physikal. Chem.*, XLIX, 298, 1904.
2. *Wied. Annalen*, XLIV, 318, 1891.
3. *Ber.*, XXII, 1764, 1889.

FIG. 2.—PRESSURE IN MM. OF MERCURY.

III. SOLUBILITY OF CARBON MONOXIDE

The gas was prepared by heating pure potassium ferrocyanide with concentrated sulphuric acid, and precautions taken to free it from air. The solubility in pure water was found to be 0.0231. Winkler¹ found 0.02338.

TABLE III. Solubility of Carbon Monoxide in blood and serum

			Natural ox blood.		Density 1.061			
Pressure in mm.	...	...	751	944	1,144	1,371	1,434	1,528
Solubility	...	...	0.0979	0.0828	0.0745	0.0658	0.0657	0.0641
			Deaërated ox blood.		Density 1.061			
Pressure in mm.	...	...	751	871	1,056	1,140	1,274	1,543
Solubility	...	...	0.0949	0.0826	0.0746	0.0710	0.0661	0.0582
			Deaërated ox serum.		Density 1.028			
Pressure in mm.	...	...	754	859	1,061	1,243	1,372	1,509
Solubility	...	...	0.0014	0.0035	0.0051	0.0066	0.0078	0.0086

See also Figs. 2 and 3.

IV. SOLUBILITY OF NITROUS OXIDE

The gas was prepared by heating pure ammonium nitrate, and was freed from air and purified by passing through solutions of potassium hydroxide and ferrous sulphate. The solubility in pure water was found to be 0.592. Geffchen² found 0.594.

TABLE IV. Solubility of nitrous oxide in blood and serum

			Natural ox blood.		Density 1.065			
Pressure in mm.	...	...	745	854	1,012	1,152	1,277	1,408
Solubility	...	...	0.521	0.530	0.539	0.544	0.547	0.548
			Natural ox serum.		Density 1.025			
Pressure in mm.	...	...	737	846	925	1,036	1,169	1,402
Solubility	...	...	0.517	0.509	0.513	0.519	0.524	0.528

See also Fig. 2.

1. *Ber.*, XXXIV, 1408, 1901.

2. *Loc. cit.*

V. SOLUBILITY OF NITROGEN

The gas was prepared by heating a mixture of sodium nitrite and ammonium chloride, and was carefully purified. Its solubility in pure water was found to be 0.0154. Bohr and Bock¹ found 0.0164.

TABLE V. Solubility of nitrogen in blood and serum

			Deaërated ox blood. Density 1.066					
Pressure in mm.	...	...	757	979	1,089	1,241	1,367	1,564
Solubility	...	...	0.0041	0.0080	0.0108	0.0135	0.0143	0.0165
			Deaërated ox serum. Density 1.028					
Pressure	...	...	734	891	1,056	1,250	1,368	1,529
Solubility	...	...	0.0008	0.0014	0.0020	0.0034	0.0047	0.0065

See Fig. also 2.

FIG. 3.—PRESSURE IN MM. OF MERCURY.

DISCUSSION OF RESULTS

It will be seen from an examination of the data obtained that the solubility phenomena observed are of two kinds. On the one hand it will be seen that the solubility of carbon dioxide, oxygen and carbon monoxide in blood is greater than in water; but that the solubility diminishes at a decreasing rate as the gas pressure is increased. A similar behaviour is

1. *Loc. cit.*

found with respect to the solubility of carbon dioxide in serum. On the other hand, it will be noticed that the solubility of nitrous oxide and nitrogen, both in blood and in serum, is less than in the case of pure water. Moreover, it will be observed that in these cases the solubility curve rises with increase of pressure, so that at high pressures it may even rise above the curve for pure water, as in the case of nitrogen and blood.

With the single exception of carbon dioxide in serum, the solubility of all the other gases is less in serum than in water, and the solubility-pressure curves are the same in type, and roughly parallel to one another. The behaviour is quite analogous to that found previously in the case of the solubility of carbon dioxide and nitrous oxide in presence of various colloids between which and the gases chemical combination seemed excluded. Quite analogous also is the rise in the solubility-pressure curve.

Since in these cases the diminished solubility of the gases in serum as compared with that in pure water appears to exclude the idea of chemical combination, and since the similarity of the solubility curves seems to point to the absence of any marked specific action, one is led to the conclusion that the continued rise in the solubility-pressure curve is to be ascribed to some physical interaction, such as surface condensation or adsorption. We are not concerned at the present moment with the real nature of these physical actions, and merely wish to indicate that the influence producing the rise in the solubility-pressure curve is most probably not a chemical combination in the usually accepted sense.¹

The rise in the solubility-pressure curve must, we believe, be ascribed to the influence of the colloidal material in the serum. Although the solubility of carbon dioxide has been determined in solutions of different salts and non-electrolytes,² in no case has an ascending solubility curve been obtained except in the case of colloiddally dissolved substances.

We find also that these different solubility-pressure curves follow the general adsorption formula $C_2^4/C_1=K$, where C_2 represents the gas concentration in the liquid, and C_1 the concentration in the colloid phase. This is shown by the following table, which refers to the curve for carbon

1. Moore and Bigland, *Bio-Chemical Journal*, V, 64, 1910, suggest the term *colloidal reaction* to a chemical process where a colloid takes part in a reaction, and reserve *adsorption* for the purely physical process. In the absence of specific chemical action, the behaviour observed here would be regarded as due to *adsorption*.

2. A number of these determinations have been made in this laboratory by Mr. B. Shen, but the results have not yet been published.

monoxide and deaërated serum. In making the calculation it has been assumed that the gas absorbed at 750 mm. pressure represents C_1 ; that is, it is assumed that no adsorption occurs at this pressure. This assumption is no doubt wrong, but the error introduced will affect only the absolute value, not the constancy of K .

Pressure	Solubility	C_1	C_2	$\frac{C_2^4}{C_1} \times 10^{10}$
750	0.0014	—	—	—
850	0.0032	0.000251	0.000196	0.058
950	0.0042	0.000437	0.000219	0.052
1,050	0.0051	0.000639	0.000242	0.053
1,150	0.0060	0.000810	0.000265	0.057
1,250	0.0070	0.001151	0.000288	0.060
1,350	0.0078	0.001421	0.000311	0.066

Similar series of values are obtained in the case of the other curves, with the single exception of that for nitrogen in deaërated serum, in which case there is a gradual fall in the value of C_2^4/C_1 . The fact that the nitrogen curve is of the same type as the others indicates, however, that the gradual fall in the value of C_2^4/C_1 is probably due to experimental errors or to the fact that a slightly different value should be chosen for the index of C_2 .

Whereas, therefore, we believe that the solubility relations in the case of oxygen, carbon monoxide, nitrogen and nitrous oxide in serum, and of the last two gases also in blood, are explicable on the basis of adsorption, no such explanation is sufficient to account for the absorption of oxygen, carbon monoxide and carbon dioxide in blood, and of the last-mentioned gas also in serum.

In these cases the solubility of the gases is markedly greater than in water, and the solubility curves *fall* with increase of pressure. To explain such curves as these requires something more than an assumption of surface adsorption.

With regard to the absorption of oxygen by blood two views are held. On the one hand there is the view, held by the majority of physiologists,¹ that the absorption of oxygen by blood is due mainly to the formation of one or more dissociable compounds; on the other hand, there is the view, represented chiefly by Wo. Ostwald,² that the chief factor concerned is adsorption, or a surface action. As already pointed out, this view was

1. See articles by Bohr, already referred to; also Moore and Bigland, *Bio-Chemical Journal*, V, 65, 1910. Barcroft and Camis, *Jour. Physiology*, XXXIX, 130, 1909.

2. *Loc. cit.*

also suggested by Findlay and Harby to explain the solubility of carbon dioxide in blood, but the investigation by the present authors of the influence of colloids on the absorption of gases, has led us to abandon this view, and to accept the 'chemical' explanation. Adsorption, we believe, does exercise an influence, but this influence appears to be of secondary importance.

It is already well known that as the oxygen-pressure increases, the absorption gradually decreases, and that between the pressures of 150 and 760 mm. only a very small extra amount of oxygen is taken up by blood (Bohr). From this behaviour Bohr concluded that the maximum degree of combination of oxygen and haemoglobin had occurred at pressures of about 150 mm.

From the curve shown in fig 3, it will be seen that although the solubility of oxygen in blood is greater than in water, it decreases as the pressure is raised.¹ The behaviour is, however, quite explicable, provided we assume, and the assumption seems justified by the work of Bohr and others, that the 'chemical combination' is practically complete at the initial pressure of 760 mm. In this case the greatest volume of gas (measured under the particular pressure employed) would be absorbed initially. As the pressure is increased further absorption would be (practically), in accordance with Henry's law, influenced to a certain extent by adsorption; but as the *amount* of combined gas remains practically constant, there would be a decrease in the total volume of gas absorbed, the volume always being measured under the pressure at which the solubility is determined.

That this explanation is at least a plausible one is shown by the following calculation. If the increase in the solubility of oxygen in blood as compared with water, be due principally to chemical combination, then the difference between the solubility in water and in blood at atmospheric pressure should give approximately the amount of oxygen that is chemically combined. Then, knowing the volume of gas absorbed by water, and that absorbed by an equal volume of blood at atmospheric pressure, the volume of oxygen absorbed by blood at any other pressure should be given by the formula—

$$V = \frac{v_1 \times p_0}{p_1} + v_2 + v_3$$

Here v_1 is the volume of gas chemically combined in 1 c.c. of blood at the initial pressure p_0 ; p_1 is the pressure at which the solubility is to

1. The *solubility* decreases, but of course the actual *amount* absorbed increases.

be determined; v_2 is the volume of gas dissolved by 1 c.c. of water at the initial pressure, and v_3 is a volume correction for gas adsorbed.

Thus, in the case of oxygen and natural ox blood, the volume of oxygen combined in 1 c.c. of blood is equal to $0.1180 - 0.0294 = 0.0886$ c.c. Then the calculated solubilities at different pressures will be as follows:—

Pressure	$\frac{v_1 p_0}{p_1}$	v_2	v_3	V	
				Calculated	Found
756	0.0886	0.0294	—	—	—
918	0.0730	0.0294	0.002	0.1044	0.1054
1,061	0.0633	0.0294	0.003	0.0957	0.0957
1,243	0.0538	0.0294	0.005	0.0882	0.0861
1,342	0.0498	0.0294	0.0065	0.0857	0.0832
1,481	0.0453	0.0294	0.007	0.0817	0.0779

The value of v_3 was taken from the solubility curve of oxygen in 'natural' serum.

The agreement shown in the above table between the solubility values calculated and found, may be regarded as very satisfactory.

The chemical explanation of the absorption of oxygen by blood is supported also by the fact that the solubility curve for oxygen and blood is of precisely similar form to that for carbon monoxide and blood. That in this case there is chemical combination between the carbon monoxide and the haemoglobin has probably never been called in question.

Just as we conclude that the increased solubility of oxygen in blood is mainly due to chemical combination, so also must we attribute the increase in the solubility of carbon dioxide in blood and also in serum, to the same cause. This view is in harmony with the experimental work of several physiologists.¹ It is true that several investigators² have, by electrical measurements and by means of indicators, obtained results which point to the blood serum being 'water-neutral,' but in view of other experimental work it seems certain that blood and serum do have power to combine with carbon dioxide,³ and the results obtained by us certainly strengthen the chemical explanation of carbon dioxide absorption.

It may perhaps be recalled that the solubility measurements with oxygen were carried out only with 'natural' ox blood, as indeed has

1. See Boycott and Chisholm, *Bio-Chemical Journal*, V, 23, 1910.

2. Hoeber, *Pflügers Archiv.*, XCIX, 572, 1903; Farkas, *ibid.*, XCVIII, 551, 1903; Friedenthal, *Verworn's Archiv. f. allgem. Physiologie*, IV, 44, 1904; van Westenryk, *Arch. exp. Path. Pharm. Suppl.*, p. 517, 1908.

3. Reference may be made to the work of Siegfried, *Zeitschr. physiolog. Chem.*, XLIV, 85, 1905; XLVI, 491.

been customary with other investigators. Although we regret that we did not also determine the solubility in deaërated blood, we do not think that the use of 'natural' blood invalidates in any way the conclusions we have arrived at. Our main purpose was to determine the *form* of the solubility-pressure curve, which our previous investigation had shown us to be of importance; but although higher values would have been obtained for the solubility of oxygen in deaërated blood, the experiments with carbon dioxide and carbon monoxide show that the *form* of the curve would still have remained the same.

It ought perhaps also to be pointed out that the conditions under which we worked (comparatively high pressures), were very different from those under which absorption of oxygen or of carbon dioxide takes place naturally. Whether, at lower pressures, the relative importance of chemical combination and of adsorption might alter, it is not possible at present to say. So far the solubility of gases in presence of colloids at pressures below atmospheric pressure, has not been determined. We hope, however, to extend the investigation in this direction.

SUMMARY

1. The solubility of oxygen, carbon dioxide, carbon monoxide, nitrous oxide, and nitrogen in ox blood and ox serum has been determined at a temperature of 25° and under pressures varying from 760 to 1,400 mm.

3. The solubility of oxygen, carbon monoxide, and carbon dioxide in blood, and of carbon dioxide in serum, is greater than in water; the solubility of nitrogen and nitrous oxide in blood and in serum, and of oxygen and carbon monoxide in serum is less than in water.

3. Where the solubility is increased, the solubility curves fall with increasing pressure; where the solubility is diminished, the solubility curve rises with increase of pressure.

4. The increased absorption of oxygen, carbon monoxide and carbon dioxide in blood, and of carbon dioxide in serum, is to be ascribed in the main to chemical combination.

5. In the cases of diminished solubility, the rise in the solubility curve is to be ascribed to adsorption.