ON THE CAPACITY OF BLOOD AND HÆMOGLOBIN TO UNITE WITH CARBON DIOXIDE. By GEORGE A. BUCKMASTER.

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Among the early workers on the blood-gases Setschenov was the first to explicitly state that the proteins of the blood,—paraglobulin, serum albumin and hæmoglobin,—were capable of forming some kind of combination with carbon dioxide. As far as the corpuscles of the blood are concerned, Setschenov considered that some carbon dioxide directly united with hæmoglobin and some with the bases which are present, but he stated that the greater part is fixed to the hæmoglobin and not to the alkali. This theory has received but little attention and certainly little support from other physiologists. It is not even mentioned in Gamgee's *Physiological Chemistry of the Animal Body*. According to Preyer, a 0·8 % solution of hæmoglobin crystals absorbed less carbon dioxide when saturated with this gas than an equal volume of water. N. Zuntz, though he confirmed the experiments that pure watery solutions of hæmoglobin take up considerably more carbon dioxide than could be accounted for by the water, said that it was unnecessary to examine the theory of Setschenov critically, since there were facts which showed that hæmoglobin could, on the most liberal estimate, unite with only a small fraction of the carbon dioxide which the blood corpuscles take up at a high partial pressure. The work of Zuntz and those who have collaborated with him has led to the belief that hæmoglobin is a comparatively unimportant constituent of the blood, so far as it confers on that liquid a capacity for fixing carbon dioxide. Direct experimental evidence of a probable relationship between hæmoglobin and carbon dioxide has been given by the absorptio-metric measurements carried out by Bohr(1) and Severin Jolin(2)

Loewy has summarised the present position as to the partition of carbon dioxide in normal blood. In 100 c.c. of blood, the red corpuscles

with a content of 14 % of hæmachrome, are capable of uniting with 7·5 c.c. of carbon dioxide provided Bohr's evidence for a union of hæmoglobin with carbon dioxide is convincing. Colloidal indiffusible substances in the blood account for 11·8 c.c. of carbon dioxide, and the bicarbonate system for 18·8 c.c.

In this paper it is not my intention to criticise views which have been held as to the relations which may obtain between carbon dioxide and any constituents of the blood, but some of the considerations which originated the experiments about to be described may be mentioned. It is well known that outside the body, blood can actually take up more than its own volume of carbon dioxide. For example, 100 c.c. of the sedimented corpuscles of the blood of the dog can take up 222 c.c. (Frédéricq), and of the horse 307·9 c.c. of this gas when saturated with carbon dioxide (Zuntz). The oxygen-expelling power which carbon dioxide exerts on oxy-hæmoglobin, which is shown on the dissociation curves of blood and solutions of hæmoglobin, both by Bohr and by Barcroft, has been held by the latter to be due to the acid properties of solutions of the gas. But this fact might be compatible with the view that one gas may actually displace the other provided the mass of one was sufficiently great.

The mean carbon dioxide content of the arterial blood of normal dogs was found by Pflüger in 27 experiments to be 38·1 c.c. per 100 c.c. of blood. The arterial blood of normal cats in 17 experiments contained 38·43 c.c. per 100 (Gardner and Buckmaster(4)). The blood in asphyxia has been frequently analysed. The average figures of 25 analyses give only 49·53 volumes per cent. But blood while circulating in the unasphyxiated animal before there is any dyspnœa at all, can and often does hold more carbon dioxide[1]. The results of Yandell Henderson and Scarborough(3) show this. In 45 analyses of the *arterial* blood of dogs under anæsthesia induced by ether and chloroform, where there was no exposure of the viscera and while the breathing was quite natural, amounts in excess of the published figures for the blood in asphyxia were often found. A large series of blood-gas analyses by Gardner and myself have also shown that in the unasphyxiated animal the venous blood could hold large quantities of carbon dioxide—at times nearly double the quantity of that found in arterial blood as is seen in the following table:

[1] Recent papers by Rasmussen (*Amer. J. of Physiol.* **39. 41.** 1916) show that the venous blood of the torpid woodchuck may contain over 100 vols. p.c. of carbon dioxide. The average value during hibernation is 88·2 %.

c.c. of carbon dioxide at 0° C. and 760 in 100 c.c. of blood.

Henderson and Scarborough	Buckmaster and Gardner	
Arterial blood of dogs	Venous blood of cats	
63·1	50·9	51·24
56·3	53·33	53·89
50·4	52·73	71·55
	53·63	

It would seem from these figures that some vehicle or vehicles for the assumption of carbon dioxide which is not already saturated with the gas must exist in the circulating blood. Since at 37° C. sodium carbonate completely changes to bicarbonate at a carbon dioxide pressure of 12·5 mm. of Hg, and three-fifths of the carbonate is bicarbonate at 0·2 mm. pressure, the blood could only take up excess of this gas as bicarbonate on the assumption that there was either a considerable addition of carbonate, or that the indiffusible colloids present were capable of doing this. But since rapid fluctuations in the salt content of the blood do not, and indeed, could not take place normally, it is difficult to consider anything of a diffusible nature as conferring upon blood an augmented capacity for the absorption of carbon dioxide. The largest fluctuations in the proteins are seen in acute septic cases which are uniformly fatal. From 10·5 %, the blood proteins may fall to 6·5 % (E. Grawitz); but this variation does not compare with what is known as to the wide variations in the quantity of the colouring matter. In other words, just as the oxygen capacity of blood is known to be related to the quantity of hæmoglobin, I proposed in the following experiments to ascertain how far the quantity of pigment could affect the uptake of carbon dioxide.

In all experiments, freshly defibrinated ox-blood was employed. Though not taken with strict aseptic precautions the blood was received into a sterile bottle and defibrinated with a bunch of sterile wires. Not later than an hour after defibrination, the blood was pipetted into sterile tubes and centrifugalised. The deposit of corpuscles was then dialysed in the cold store at 0°–1° C. To effect efficient dialysis, I used six to eight paper soufflé cases, each holding a deposit of about 0·5–1 cm. deep. These were floated in tall beakers holding about 200 c.c. of distilled water, which was renewed four or five times in 24 hours. The dialysis continued for 2–8 days until only the faintest trace of the precipitate appeared in the dialysate five minutes after the addition of silver nitrate. The corpuscular deposit became laked. It was perfectly

transparent, but to avoid any slight precipitate of cell globulin which might exist, a minute quantity of solid sodium chloride was added before the dialysed corpuscles were treated with carbon dioxide.

Saturation with carbon dioxide. The dialysed corpuscles as such, or after suitable dilution with water, were either saturated with carbon dioxide for a given time in a vessel such as that employed by Exner, or in a special absorbing wash-bottle in which the bubbles successively rise through a spiral channel about 84 cm. long. The saturations were always carried out at a uniform temperature in a thermostat kept at 38° C. The carbon dioxide used was collected from a cylinder of the gas and stored in a gasometer. Analyses of this from time to time gave values between 92·9 and 94·2 % of carbon dioxide. The admixed gas was air. A small wash-bottle enabled the rate of flow to be judged, and before saturation, the sampling tubes and vessel for saturation were filled with boiled out water, which was then completely displaced by the gas. The dialysed blood was then introduced into the saturation vessel and saturated for a variable length of time. I have shown that when the saturation goes on for some four or five hours, an acid hæmatin is produced. The average time for saturation was 1 to 1·5 hours, and though it is impossible to exclude the possibility that a minute amount of acid hæmatin was produced, it is improbable that this occurred since the hæmoglobinometer readings were quite unaffected.

When saturated at pressures below 93 % of an atmosphere of CO_2, the saturation vessel varied in different experiments. Sometimes it was a closed bulb holding 179 c.c., or a wash-bottle of capacity 290 c.c.; such vessels having been filled with water, this was displaced by the gas mixture. About 50 c.c. of dialysed corpuscles were then introduced, and the carbon dioxide mixture from a 10-litre bottle run through for half an hour at room temperature. The mean carbon dioxide values of two gas-sampling tubes on the inflow and outflow was taken, giving the carbon dioxide content of the saturation vessel. The blood and gas mixture was gently shaken at 38° C. by an arrangement connected with a small turbine.

For determinations with known pressures of carbon dioxide I have, though not in the early experiments, used the following apparatus (see Fig. 1), since objections have been made to the results obtained by ærotonometer methods on the ground that blood, as it streams into the apparatus, may so change as to give fallacious and inaccurate measurements of tension. Bulbs *B*, *A* and *C* have respectively capacities of

36 c.c., 350 c.c. and 50 c.c. By lowering and raising the mercury reservoir, *C* is completely filled from *D*, and the contents clamped off. *D* is cut off by a clamp and the whole system I, *B*, *A*, II filled with water. This is displaced with gas from the gas-holder and a stream of gas flows

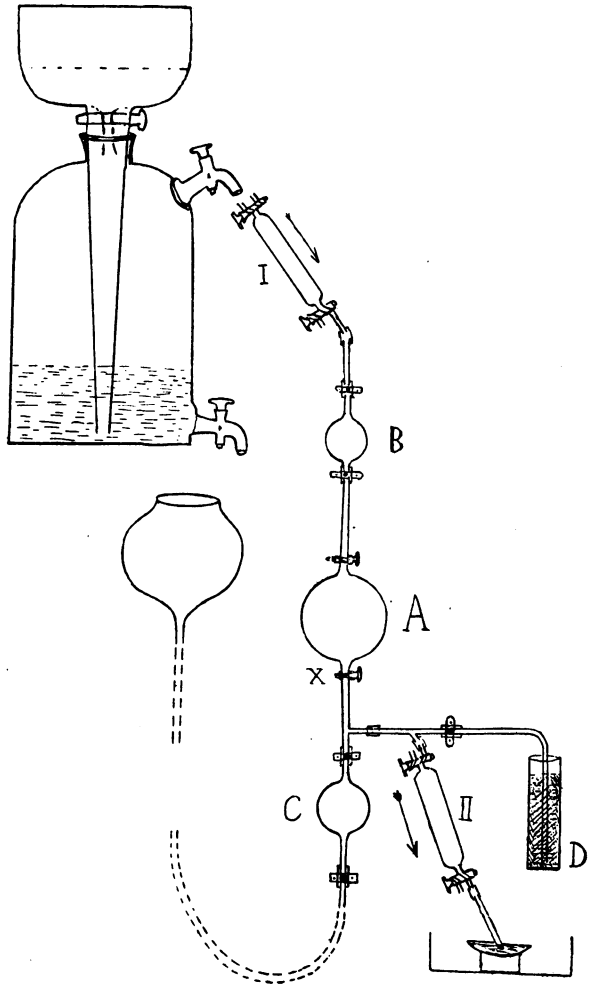

Fig. 1.

through for some minutes. The mean of the analyses of the sampling tubes I and II gives the carbon dioxide pressure in *A*. The blood from *C* is passed into *A*. At *X* is a tail-tap out of which a little blood flows before it is caused to enter *A* and displace gas through *B*. The

taps in connection with *A* are now shut, the vessel is disconnected and gently shaken continuously in a thermometer at 38° C. by means of a small turbine. *A* is finally replaced as in the figure; the blood is passed into *B* which contains carbon dioxide at original pressure. This bulb is now clamped off and connected to the pump for evacuation of gases, any tubes or junctions being filled with gas-free water.

Evacuation of the gas. This was carried out as described and figured in a former paper (7). A modification of Leonard Hill's pump was so arranged that the gases could be directly passed into the gas analysis apparatus devised by J. S. Haldane. In some of the earlier experiments, the evolved gas was collected in an eudiometer tube which was transferred with an iron spoon to a well-shaped pneumatic trough full of mercury, and the absorption determinations made with a small amount of 40 % potash. This method cannot be regarded as accurate. The error is at least double the value, 1 % given by Leonard Hill.

The hæmoglobin values were obtained with the Gowers-Haldane instrument, using the residual blood in bulb *A* which was not required for evacuation. The standard tube of carbonic oxide hæmoglobin I made from ox-blood of known oxygen capacity and I have taken 13·8 % of hæmoglobin as corresponding to 18·5 c.c. percentage oxygen capacity equal to 100 on the hæmoglobinometer scale. The accuracy of the standard was verified from time to time by testing this against fresh ox-blood the oxygen capacity of which was ascertained in the pump.

Action of carbon dioxide on blood and solutions of hæmoglobin.

Preyer(8) and Ray Lankester(9) have shown that the prolonged passage of a stream of carbon dioxide through solutions of hæmoglobin or blood causes a change in tint of the solutions and the appearance of an absorption band in the red, λ 630[1]. It is easy to confirm this. The solutions are more acid. The hæmoglobin is to a variable extent converted into acid hæmatin. Though this substance has been regarded as methæmoglobin, this is improbable. In no case will the spectrum of methæmoglobin which has been produced by the action of iodine, glycerine, ferricyanide of potassium, hydroxylamine or permanganate of potassium change when the solutions are rendered gas-free in the pump. But the carbon dioxide product when evacuated definitely changes. The band in the red disappears and the single

[1] Menzies has shown that this band tends to shift towards λ 642 and λ 650 with increasing degrees of acidity produced by organic and mineral acids. This *Journal*, 17 423. 1894–95.

band of reduced hæmoglobin appears. On re-oxygenation and re-saturation with carbon dioxide, absorption band λ 630 reappears, to again disappear when evacuated by the pump. Since these changes can be repeated several times, it is possible that the effect of the gas does not affect the structure of the hæmoglobin molecule to an appreciable extent. The removal of acidity by physical means restores the pigment to its normal condition, provided that the original saturation with carbon dioxide has been carried out at a temperature of 0°–4° C. and has not been too prolonged. For at temperatures of 20° C. to 38° C. the blood outside the body at once begins to spontaneously undergo changes which are permanent.

The quantities of carbon dioxide absorbed by blood is seen from the following table. The analyses were made in an eudiometer.

50 c.c. of fresh defibrinated blood or solutions of this saturated with 94·5 % of carbon dioxide at 38° C.

Dog's blood.

		Saturation in hours	Bar. P.	Hb. %	c.c. of CO_2 in 100 c.c. at 38° C. and P.
A.	Diluted with distilled water	4	753	2·0	112·12
B.	„ „ „ „	1	753	2·0	113·1
C.	Laked deposit of corpuscles	1	753	11·8	126·2
D.	Fresh defibrinated blood	3	751	12·3	130·0
E.	„ „ „	5	761	14·3	152·0

Ox blood.

F.	Fresh defibrinated	1	758	9·3	120·0
G.	„ „	1·5	742	10·7	125·0
H.	„ „	1·5	742	10·7	124·2
I.	„ „	1	753	11·4	131·16
J.	„ „	2	753	12·67	133·1
K.	„ „	1	749	20·7	146·1

It is evident that blood can absorb quantities of carbon dioxide. It is not necessary to indicate the amount absorbed by water in individual experiments but a subtraction of 52·44 c.c. in each experiment would be correct for expt. E, and in excess for all the others where the 100 c.c. of blood is reckoned as 100 of water. For example, in expts. E, D, H, J and K, apart from solution in water, blood would hold 99·56 c.c., 87·56 c.c., 81·66 c.c. and 93·66 c.c. of carbon dioxide. Within limits, the amount of gas absorbed would appear to rise and fall with the quantities of hæmoglobin present, and therefore the quantity of the pigment is a determining factor in this matter.

Absorption of carbon dioxide by hæmoglobin (hæmachrome).

From Abderhalden's figures(10) the composition of the corpuscles of the ox would give in 100 grams:

Hæmoglobin	31·67 grms.
Proteins	6·42 „
Alkali available for the protein and carbon dioxide	0·147 „

In dialysed blood, Hb/P is raised, since no matter how thoroughly centrifugalisation has been carried out, some serum, the protein content of which in the ox is 7·25 %, adheres to the corpuscles.

If the protein values of the corpuscles given by Abderhalden are examined, the percentage is very high in the ox, exceeding that of eight other animals, where values as low as 0·9, 0·5, 1·2, 2·6 and 3·7 were obtained. Further, in the blood of the *same* animal, the protein figures for the sheep are 7·84 and 3·79, while in two dogs the protein content differs by nearly 100 %. The method of determination of protein was an old one, proposed in 1876 by Hoppe-Seyler. It is doubtful whether the value is correct, since the corpuscles of the steer contain 4·6 of protein, compared with 6·42 for the ox.

The following six determinations of the dried residue of dialysed corpuscles give the total solids of the corpuscles, *minus* salts, which amount to 0·75 %. By deduction of the hæmoglobin the value of the other colloids is obtained, since the cholesterin-lecithin content together is only ·06 %. The differences between the hæmoglobin content and the total solids will indicate the protein content within ·06 %. The total solids vary, of course, with the quantity of water entering the dialyser.

Total solids	Hæmoglobin	Protein
13·61	9·66	3·95
13·65	9·61	4·04
11·12	4·1	6·02
11·85	6·56	5·29
13·72	8·87	4·85
12·12	7·02	5·10
76·07		29·25

The average protein content will, therefore, be 4·87 %. In the subsequent calculations, so as not to unduly magnify the absorption power of hæmoglobin, I have taken the average protein content of the corpuscles of the ox, *plus* the adherent serum, as 5·0 % of the solids of the corpuscles, which possess 31·67 % of hæmoglobin. The ratio of hæmoglobin to protein will be 1/0·157.

The following table gives the results of 23 experiments made by saturating the hæmoglobin of blood with 93–94·2 % carbon dioxide at 38° C. In the sixth column of figures the values are purposely calculated for 100 % saturation. The figures in the last column therefore are the *minimal* values showing the quantity of gas which the colloids of the blood can take up.

c.c. of carbon dioxide in 100 c.c. of liquid reckoning 94 % CO_2 = 100 %.

	Hours sat.	Bar. P.	Hb. %	Total 0° 760	Absorbed by water at 38° and P.	Absorbed 0° C. and 760 P.	United with proteins Hb. + P.
A	2	741	0·8	51·0	52·45	45·32	5·68
B	1	752	1·38	67·0	54·25	47·06	18·94
C	1·5	761	2·12	53·1	54·3	47·7	8·4
D	1	752	2·5	57·0	53·54	46·5	10·5
E	3	760	4·1	73·0	53·3	47·3	25·7
F	1	749	4·1	71·0	52·4	45·3	25·7
G	1·5	761	4·25	61·0	53·5	46·06	14·94
H	1	770	4·5	75·0	53·6	47·4	25·6
I	1	747	5·56	61·0	51·5	44·4	16·6
J	1	742	6·05	60·5	50·9	42·6	17·9
K	1	748	6·2	77·1	51·22	43·25	33·85
L	1·5	761	8·3	82·5	50·9	44·74	57·76
M	0·75	742	8·8	89·2	48·24	41·16	48·04
N	1·5	742	8·8	89·2	48·24	41·16	48·04
O	2·5	760	8·8	84·6	50·61	44·4	40·2
P	3	760	9·6	89·56	50·16	44·99	44·57
Q	3	760	9·6	88·03	50·16	44·99	43·04
R	2	751	11·8	87·74	48·40	41·97	45·77
S	3	751	11·8	86·17	48·40	41·97	44·20
T	1	752	13·8	94·7	47·33	41·07	53·63
U	3	767	14·0	120·0	48·15	33·83	116·17
V	1	770	18·0	150·2	46·05	41·37	148·83
W	0·75	754	20·0	118·3	44·03	33·17	85·13

It is evident that salt-free blood can absorb quantities of carbon dioxide and that within limits the capacity to do this augments with the percentage of hæmoglobin. I have thought it advisable to give not selected but the total experiments. Six out of 23 experiments—B, G, I, J, O and V—are, I think of doubtful accuracy. These were early ones and the measurements were made in an eudiometer. I have considered the extra absorbed gas to be united with both the blood pigment and the proteins, though in many experiments, the object of which was to ascertain whether the proteins of serum, egg white and whey had the capacity of absorbing dioxide, I have not found much

evidence to indicate that proteins free from salts can absorb this gas to any marked extent. But the figures given by Nagel(11) for serum, though he does not draw this inference, appear to me to show that the absorption of carbon dioxide by proteins is possible. This view is generally held to be the case. Therefore, in the following table taking the value of hæmoglobin to proteins in dialysed blood corpuscles as 1/0·157, I have assumed that proteins can absorb carbon dioxide to an extent equal to that of hæmoglobin. Even with this admission, the blood pigment absorbs quite large amounts of carbon dioxide.

Hb. %	Total CO_2 in c.c. 100 at 0° and 760 absorbed by Hb. + P.	Absorbed by proteins	Absorbed by hæmoglobin	1 gr. Hb. absorbs
2·5	10·5	1·427	9·073	1·1
4·1	25·7	3·49	22·721	1·2
6·2	33·85	4·73	29·12	1·13
8·8	48·04	6·52	41·52	1·1
14	116·17	14·27	101·8	1·14
18	148·83	20·16	128·67	1·15

The following results also show the influence of varying quantities of the same deposit of dialysed corpuscles added to water in different percentages.

Deposits of centrifugalised corpuscles were dialysed at 0° C. for 4 days:

Hæmoglobinometer = 161 = 22·2 % haemoglobin

Total solids	28·35 %	
Hæmoglobin	22·2 „	
Proteins	= 6·15 „	

of this deposit, three solutions were made as follows:

A	50 c.c.	corpuscles	+	65	distilled	water
B	25 „	„	+	65	„	„
C	10 „	„	+	65	„	„

Each of these was saturated with 96 % carbon dioxide for 1·5 hours at 38° C. and then evacuated in the pump with the following results:

	B.P. mm. Hg.	Hb. %	Total 0° and 760	Abs. at 38° C. and P.	Abs. at 0° C. and 760		Difference
A	767	9·6	94·0	50·7	44·5	=	49·5
B	767	6·1	72·0	52·6	46·2	=	25·8
C	767	3·6	62·0	53·4	46·8	=	15·2

Calculating from this and excluding the proteins, the following values are obtained for 1 grm. of hæmoglobin at 0° and 760

A = 5·1 c.c. B = 4·09 c.c. C = 4·2 c.c.

Saturation of dialysed corpuscles with carbon dioxide at varying pressures.

The following tables give a few examples of the absorption of carbon dioxide by hæmoglobin at known pressures of the gas at 38° C.

I.

Laked dialysed corpuscles of ox-blood saturated at 38° C. 32 c.c. taken for evacuation in pump.

	Duration of saturation in hours	P.	Hb. %	Pressure of CO_2 in mm. Hg.	Total CO_2 0° and 760	CO_2 in c.c. physically absorbed at 38°	CO_2 in c.c. physically absorbed 0° and 760	Absorbed by Hb. + P.	Absorbed by Hb.	1 gram of Hb. absorbs c.c. of CO_2 at 0° and 760
A	0·75	760	2·5	105·6	3·81	2·4	2·14	1·67	= 1·44	0·56
B	1·0	760	4·17	104·3	4·48	2·1	2·24	2·60	= 2·24	0·56
C*	2·0	760	11·04	103·05	8·42	1·3	1·21	7·21	= 6·23	0·56

* I discovered later that the sampling tube leaked in this experiment when adjusted to the gas analysis apparatus, 103·05 mm. Hg is therefore too high, probably nearer 90 mm.

II.

	Saturation in hours	Hb. %	Pressure of CO_2 in mm. Hg.	c.c. of CO_2 per 100 c.c. of blood total	absorbed	Difference
A	1	2·8	70·6	20·02	6·0	14·0
B	1	14·0	70·6	37·3	4·88	32·42
C	1	8·6	140·0	30·1	17·1	12·9
D	1	11·8	140·0	40·1	17·1	22·0

The results of these tables show definitely that the total carbon dioxide which can be absorbed at a pressure of 104 mm. Hg rises and falls with the hæmoglobin percentages, and from these experiments the conclusion may be drawn that hæmoglobin is capable of taking up considerable amounts of carbon dioxide at pressures between 760 mm. and 70 mm. Such pressures do not occur normally in the body, and statements as to the behaviour of the blood pigment to this gas at lower pressures will not be given until the question of the degree of absorption of carbon dioxide by salt-free proteins other than hæmoglobin is definitely answered. It would seem that until this information exists it would be premature to attempt to assign values to the quantity of gas actually taken up by dialysed blood at different pressures of the gas, especially since Bohr's figures for this show wide variations. Further experiments may show that all the proteins of the blood possess the property of absorbing carbon dioxide. So far I have found that none of these exhibit this feature to the degree possessed by hæmoglobin. This pigment may behave generally like other proteins, but in addition possess a specific capacity for the absorption of carbon dioxide.

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