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THE TRANSPORT OF CARBON DIOXIDE BY ERYTHROCYTES AND PLASMA IN DOGFISH BLOOD

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During the summer of 1936, unsuccessful attempts were made by J. K. W. F. and J. R. P. to measure (using the chemical method of Ferguson and Roughton, 1934) the carbamino compounds of CO_2 with hemoglobin of mackerel blood and dogfish blood. The difficulty of preparing stable solutions of hemoglobin from these bloods proved to be the most serious obstacle. An attempt was then made to apply the kinetic method of Meldrum and Roughton (1933) to dogfish blood. This too was unsuccessful because carbonic anhydrase could not be completely inactivated by cyanide as required in this method.

Some evidence as to the state of CO_2 in dogfish blood was then sought by determining the distribution of CO_2 between the erythrocytes and plasma. A few preliminary experiments revealed, unexpectedly, that the erythrocytes seemed often to contain more CO_2 than the plasma, a relationship which is the opposite to that found in mammalian blood. A continuation of these experiments, during the summer of 1937, has established the fact that there are peculiarities in the transport of CO_2 in dogfish blood, which may be of general significance.

Methods

Dogfish were used exclusively in this study as they were about the only available source of sufficient amounts of blood. Thirty of the thirty-two fish used were of the smooth variety (*Mustelus canis*). The other two were spiny dogfish (*Squalus acanthias*). Usually the blood was drawn by paracentesis of the dorsal blood vessels through the ventral body wall behind the cloaca. Aeration of the gills could be maintained throughout this procedure while the fish was held almost motionless. Heparin (15 units per cc. of blood) proved to be a satisfactory anticoagulant, but defibrination was just as satisfactory for most purposes. At first, attempts were made to use fluoride and oxalate (the

fluoride to check glycolysis). It soon appeared that both fluoride and oxalate caused progressive swelling and eventual hemolysis of the erythrocytes. This phenomena is being investigated further. Fortunately the use of fluoride proved unnecessary. When the blood was kept at 10° C., the loss in CO_2 capacity at a CO_2 pressure of about 20 mm. Hg was less than 1 vol. per cent after eight hours. Each dissociation curve was completed in less time than that.

Equilibration of the blood with gas mixtures was done by the first method of Austin et al (1922) in a water bath at a temperature of 22° C., or sometimes of 23° C. The equilibrated blood was segregated in a centrifuge tube joined to the tonometer by wide rubber tubing. The gas phase was analyzed in either a Van Slyke or a Haldane apparatus. After samples of whole blood had been removed for the various analyses (O_2, CO_2, H_2O and Cl) and the determination of cell volume, the remainder was centrifuged under oil at about 2800 r.p.m. for 30 minutes. Analyses were then made on the plasma. When the volume of packed cells was sufficient, analyses were made on them, frequently for chloride and H_2O, but only on one occasion for CO_2.

Calculations

The concentration of CO_2 in the erythrocytes was calculated by the formula

$$C = \frac{B - V \times P}{v},$$

where $C = CO_2$ in erythrocytes (v.p.c.), $B = CO_2$ in whole blood (v.p.c.), $P = CO_2$ in plasma (v.p.c.), $V =$ volume of plasma per cc. whole blood, $v =$ volume of erythrocytes per cc. whole blood. In all cases in this paper CO_2 means total CO_2.

The distribution ratio of CO_2 between cells and plasma was calculated by the formula:

$$rCO_2 = \frac{C}{P} \times \frac{Wp}{Wc},$$

where $Wp = gH_2O/100$ cc. plasma, $Wc = gH_2O/100$ cc. cells.

The distribution ratio of chloride (rCl) was calculated in a similar manner.

Sources of Error

An analytical figure for CO_2 in plasma or blood may be in error by about 0.5 v.p.c. When the CO_2 content of the blood is about 10 v.p.c. (a typical value for venous dogfish blood), such an error might

produce a large error in the calculated value of C and hence of rCO_2. For example, if the analysis of the plasma gave a result too high by 0.5 v.p.c., and the analysis of the whole blood too low by 0.5 v.p.c., an error of 38 p.c. would result in rCO_2. For this reason little importance has been attached to individual values of rCO_2. Each value of rCO_2 reported in this paper is a mean for a group of comparable determinations.

In addition to random errors, a systematic error may be inherent in the hematocrit determinations since centrifuge speeds above 3000 r.p.m. were not used. If the hematocrit numbers are systematically too high, due to failure to separate completely the plasma from the cells, the error would tend to obscure any real discrepancy between the concentra-

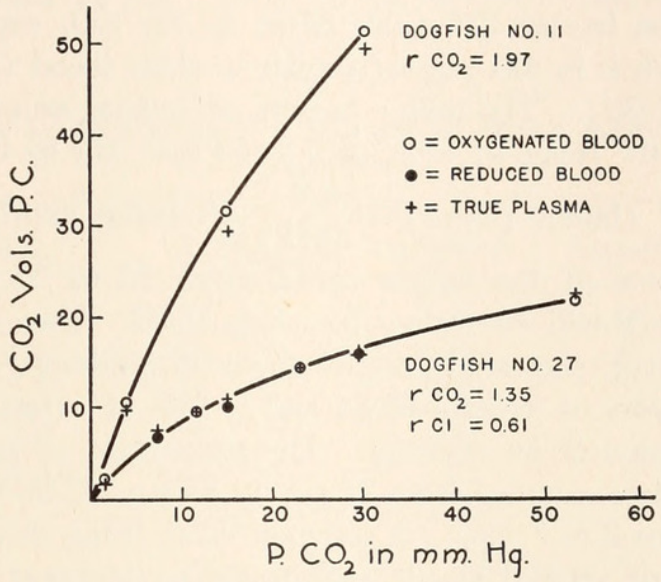

FIG. 1. CO_2 dissociation curves of whole blood and true plasma of dogfish (11) and (27).

tions of CO_2 in cells and plasma. Similarly in the estimation of chloride and water content of the cells, allowance for plasma trapped between the cells would tend to diminish the reported values of rCl and increase those of rCO_2. Thus in every case the systematic errors, if they are significant, would tend to obscure, not enhance, the discrepancies which are the main findings of this investigation.

RESULTS

The characteristics of the CO_2 dissociation curves of the whole blood will be presented first, then the data relating to the distribution of CO_2 between cells and plasma.

The CO_2 dissociation curves of dogfish blood resemble those of other vertebrates in many respects. When the log of P_{CO_2} is plotted

against the log of the CO_2 content, a straight line is obtained, except for points at very low pressures of CO_2, i.e. less than 1 mm. Hg. Since analytical errors are magnified at these low values of CO_2 and P_{CO_2}, not much significance can be attached to the discrepancies, although they might be due to carbamino compounds.

The position and slope of the curves vary greatly from fish to fish. The lowest CO_2 content at a CO_2 pressure of 30 mm. was 16 v.p.c.; the highest was 52 v.p.c. Part of this variation was due doubtless to great variation in erythrocyte volume, e.g. from 10 to 25 p.c. It was suspected at first that a seasonal factor was of importance since all of our higher values for CO_2 capacity were obtained early in the summer. A few determinations early in the summer of 1938 showed that this relationship was not invariable.

Oxygenation has no detectable effect on the CO_2 capacity of dogfish blood which is in this respect similar to skate blood (Dill, Edwards and Florkin, 1932). The buffer powers of several samples have been calculated for the range of P_{CO_2} of 5 to 50 mm. Hg by the formula of Van Slyke, B (buffer power) $= \frac{\Delta B}{\Delta \mathrm{pH}}$, ΔB being expressed in millimols/liter. Most of the values varied from 10 to 20 millimols/pH, which agrees well with the values for skate blood. Two specimens (10 and 11), however, showed values of about 90 millimols/pH.

Many samples of venous blood and a few of arterial blood were analysed immediately on removal. The percentage of saturation with O_2 varied in these samples from 25 p.c. to 75 p.c., while the O_2 capacities varied from 2 to 7 v.p.c.; a common value being about 4 p.c. No samples, even of arterial blood, were found to be more saturated than 75 p.c., perhaps because of the relatively high environmental temperature, or of the artificial conditions of aeration of the gills. Nevertheless, one may infer from these data the probable range of CO_2 content and pressure in dogfish blood. The CO_2 content of arterial blood is probably about 6–8 v.p.c. at a tension of perhaps 3 or 4 mm. Hg. The highest value found for CO_2 content of venous blood was 12 v.p.c. at a probable tension of 10 or 12 mm. Hg. Presumably, higher values than this may be attained under conditions of stress. In two specimens, numbers (10) and (11), which had steep dissociation curves, the estimated P_{CO_2} for arterial and venous blood was 2 and 4 mm. Hg respectively. The conditions in these two fish are more like those reported by Dill, Edwards and Florkin for the skate. We can substantiate the comment of these authors that the transport of CO_2 can be accomplished, with relatively small differences in CO_2 tension between venous and arterial blood, in spite of the absence of any effect of O_2 on the CO_2

capacity, because the physiological range of the CO_2 dissociation curve in these forms is on the steepest part of the curve.

Distribution of Carbon Dioxide and Chloride

Table I summarizes the results on distribution of CO_2 and Cl between erythrocytes and plasma. It is apparent that in the majority of specimens the CO_2 content of the erythrocytes per unit of water is definitely higher than that of the plasma in equilibrium with them. The chloride content, on the other hand, is definitely lower in the erythrocytes, which is what one would expect if the conditions of ionic equilibrium were similar to those in mammalian erythrocytes, and determined according to the Donnan Equilibrium. In only one specimen was rCO_2 as low as one. Even in this one, the discrepancy between

Table I

Summary of data on the distribution of CO_2 and Cl between erythrocytes and plasma or serum

Experiment	Number of analyses	Mean rCO_2	Mean rCl	rCO_2/rCl
Dogfish (23)	7	1.03	.49	2.11
Dogfish (26)	7	1.57	.66	2.46
Dogfish (27)	7	1.35	.61	2.23
Dogfish (spiny) (11)	3	1.97	—	—
Dogfish (spiny) (22)	3	1.65	—	—
Various venous bloods	11	1.46	—	—

rCO_2 and rCl is maintained by a low rCl. It should be noted too that the values of rCl are definitely lower than those found at the same pH in mammalian blood. Another point worthy of note is that no tendency was observed for the hematocrit number, rCO_2 or rCl, to vary in any regular fashion with changes in P_{CO_2} ranging from 0 to 60 mm. Hg. For this reason it is justifiable to calculate a mean value for rCO_2 and rCl for the data of one dissociation curve. It should be emphasized, however, that since the hematocrit measurements are rather crude, it cannot be concluded from these data that there is no shift in water or chloride, when CO_2 is added to dogfish blood. This point requires further investigation over wider ranges of P_{CO_2}.

Some idea of the regularity of the data may be obtained from Fig. 1 which shows the CO_2 dissociation curves of blood and true plasma of dogfishes (27) and (11). It is seen that the CO_2 contents of whole blood and plasma (and hence of cells and plasma) are practically equal in (27). When allowance is made for the lower water content of the

TABLE II

Complete data of a typical experiment on the heparinized blood of dogfish (23)

	P_{CO_2}	Blood CO_2	Plasma CO_2	Hematocrit	H_2O		Chlorides	
					Plasma	Cells	Plasma	Cells
	mm. Hg	*v.p.c.*	*v.p.c.*		*grams/100 cc.*	*grams/100 cc.*	*m.eq./liter*	*m.eq./liter*
Oxy.....	61.3	38.8	40.0	23.4		63.7	253	94
Red.....	39.6	34.7	36.3	22.7	89.6	63.7	255	83
Oxy.....	31.5	30.6	33.0	21.7	89.4		254	78
Red.....	21.0	25.3	27.7	21.8			256	74
Oxy.....	16.5	25.2	26.8	23.3			254	90
Red.....	12.0	20.3	21.3	22.9	89.7	63.5	256	83
Oxy.....	8.2	15.1	16.6	23.1			254	90
				Av. 22.5	89.6	63.6		

cells, it is evident that the concentration of CO_2 per unit of water is higher in the cells than in the plasma. A striking excess of CO_2 in the cells of (11) (a spiny dogfish) is evident. Almost identical results were obtained on (10) (a smooth dogfish). In Table II are presented the complete data of one experiment (dogfish 23). The greatest discrepancies are shown in figures for chloride content of the cells. Inhomogeneity of the samples of packed cells may be responsible for these variations.

DISCUSSION

It is evident that considerably more CO_2 is present in the red cell than can be accounted for by the combined assumptions that the bulk of the CO_2 in the red cell is in the form of free bicarbonate ions, and that the distribution of bicarbonate and chloride ions is according to the Donnan Equilibrium. If it is assumed for the purpose of calculation that the distribution ratio of free bicarbonate ions is equal to that of the chloride ions, then the extra CO_2 in the red cells (E_{CO_2}) may be calculated in approximate fashion as follows:

$$E_{CO_2} = [CO_2]\ (cells) - [CO_2]\ (plasma) \times rCl.$$

Since rCl and rCO_2 are practically constant over the range studied, E_{CO_2} increases progressively with P_{CO_2}. This would suggest that the extra CO_2 in the cells was not in the form of carbamate, since carbamino compounds of mammalian hemoglobin are present in maximal concentration at very low pressures of CO_2, and tend to decrease with increasing P_{CO_2}.

Two other possible explanations of the extra CO_2 deserve some consideration. It is possible that some combination of a non-carbamate

type may occur between bicarbonate ions and cellular proteins (hemoglobin or others). The evidence for such a compound is discussed in the review by Roughton (1935) under the heading Y-bound CO_2. The likelihood of the existence of such compounds is supported somewhat by the demonstration recently of a specific effect of bicarbonate on the O_2 capacity of hemoglobin by Sidwell et al. (1938).

The other possibility invokes the participation of the nucleus. It has been shown by Chambers and Pollack (1927) that the nuclei of starfish eggs are distinctly more alkaline than the cytoplasm (pH 7.6 as compared with pH 6.8). If this is true in the dogfish erythrocyte one would expect that, at any given pressure of CO_2, the concentration of combined CO_2 would be five or six times greater in the nucleus than in the cytoplasm. If it were further assumed that the nucleus contained little or no chloride, the discrepancy between rCO_2 and rCl could be explained, as well as the low values of rCl relative to mammalian blood. It seems impossible at the present time to decide which, if any, of these views may be correct.

In searching the literature for observations similar to ours, we found the paper of Dill and Edwards (1931) on crocodile blood. They had found an apparent excess of CO_2 in the red cells in both reduced and oxygenated blood. In two respects, however, their findings were different from ours. On reduction of the crocodile blood, the excess CO_2 in the red cell was increased enormously. This suggests that carbamino compounds of CO_2 with hemoglobin may be very prominent in crocodile blood. The other difference, which may or may not be as significant, was that the distribution ratios for chloride were more nearly similar to those in mammalian blood. If the nucleus in these cells were poor in chloride the volume of the nucleus would have to be small compared with the cell volume.

Summary

The CO_2 dissociation curves of dogfish blood (*Mustelus canis*) are in most respects like those of other vertebrates, Log P_{CO_2} plotted against Log $[CO_2]$ gives a straight line.

The CO_2 capacity is not affected by the degree of oxygenation of the hemoglobin.

The oxygen capacities in different specimens varied from 2 to 7 v.p.c.

The tension of CO_2 is estimated to vary, in arterial bloods from 2 to 6 mm. Hg; in venous bloods from 4 to 12 mm. Hg with the CO_2 contents ranging from 6 to 12 v.p.c.

The concentration of CO_2 in the erythrocytes is usually greater than in the surrounding plasma. Reasons are given for supposing the "extra" CO_2 in the red cells *not* to be carbhemoglobin. Two other possible explanations for the extra CO_2 are discussed.

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