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THE pH, CARBON DIOXIDE TENSION, AND THE HEMOGLOBIN PERCENTAGES OF VENOUS BLOOD OF VARIOUS FRESH WATER FISHES

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The carbon dioxide tension of the water has been shown to be an important environmental factor for fishes (Powers and colleagues, various publications). It has been found that the carbon dioxide tension of the blood of fishes approximates the carbon dioxide tension of the water that bathes their gills. The pH of the venous blood of certain fresh water fishes, under experimental conditions, as determined by the Beckman pH meter glass electrode was fairly constant, pH 6.20 to 7.07 over a wide range of carbon dioxide tensions, approximately .09 to 20 mm. Hg (Powers, Rostorfer, Shipe and Rostorfer, 1938). This is far beyond any carbon dioxide tension range found in natural waters under the most adverse conditions where fish are still able to survive. The pH level of the blood in the species of fishes tested, rock bass (*Ambloplites rupestris*), small mouth bass (*Micropterus dolomieu*), and yellow perch (*Perca flavescens*), in the experiments just cited, is maintained by a modification of the alkali reserve of the blood either up or down.

Following these experiments it was decided to determine the pH of the blood of different species of fishes living under natural conditions.

METHODS

The methods were outlined by the senior author and the observations were made by the two junior authors. The work was carried on for the most part at the Franz Theodore Stone

Laboratory, Put-in-Bay, Ohio. A few of the observations were made at the Zoology Laboratory, University of Tennessee, Knoxville. Fishes were taken by hook and line or seined and placed in live boxes or running water tanks. The fishes were taken from the live boxes in containers and the pH of the blood determined by means of a Beckman pH meter glass electrode as quickly as possible. In a few cases the pH of the blood was determined immediately after taking the fish with hook and line. Blood was drawn from the exposed conus arteriosus with a 5 cc. Leur syringe. Care was taken to withdraw the blood without subjecting it to a negative pressure. However, this could not always be avoided.

All blood tested was *venous* and not *arterial.*

The pH of a portion of the drawn blood was determined immediately and before coagulation took place. A second portion was centrifuged until a clear serum appeared at the top of the centrifuging tube. No anti-coagulant was used. The unmodified serum was then aerated by means of a fine pointed asperator and the pH determined as in the whole blood. Due to the small amount of blood and serum and the use of laboratory air for aeration, small errors could not be prevented. However, no corrections have been made in any of the data.

DATA AND DISCUSSION

In Table I data are arranged in order of the pH difference between the unaerated and aerated environmental water. According to the formula (—) $pH = -ne-n\log PCO_2$ (Powers, 1927, and Powers and Bond, 1927, 1928), the positive difference is the positive difference between the logarithm of the carbon dioxide partial pressure of the atmosphere and the logarithm of the carbon dioxide tension of the water. The negative difference is the negative difference as stated above. These have not been calculated in this paper since neither the value of n nor the barometric pressure were determined and, thus, the calculated per cent of an atmosphere of carbon dioxide tension below or above the carbon dioxide partial pressure of the atmosphere would be only a close approximation. Figure 1 is a graphic representation of the data of Table I.

When the data, Table 1, and the graphs, Figure 1, are examined it is found that the pH of the blood of a species of fish is fairly constant. In the carp (*Cyprinus carpio*) the extreme variation of pH is 7.19 to 7.56 or .37 pH units. The small

mouth bass has a variation of pH 6.89 to 7.55 or .66 pH units. The perch shows a pH difference of 6.90 to 7.47 or .57 pH units. The pH difference in the blood of the rock bass is 7.02 to 7.75 or .73 pH units. In Table II where data of all fish tested are given the extreme variation in the pH of the blood are the carp, .66 pH units, the rock bass, 1.04, the perch, .77, the small mouth bass, 1.11, the sheepshead (*Aplodinotus grunniens*), .18, the yellow catfish (*Amieurus natalis*), .47 and the jack salmon (*Stizostedion vitreum*,).12. These with two exceptions, the carp, 1.04 pH units, and the small mouth bass, 1.11 pH units, are well within the extreme difference in pH (cH 2.5×10^{-7}) 6.60 and (cH 3×10^{-8}) 7.52 or .92 pH units of the blood of the scup as given by Barcroft (1934).

The difference in pH units between the pH of the blood and the pH of the aerated serum in each species of fish has a greater extreme range than the pH range in the aerated serum, carp .31, rock bass .74, the perch .31, small mouth bass .54, sheepshead[1] .19, yellow catfish .43, and the jack salmon[1] .04. When averages of pH units differences between the blood and aerated serum are considered, we find the following: carp Table I .92 and Table II .87, rock bass Table I .92 and Table II .90, small mouth bass Table I 1.02 and Table II 1.03, sheepshead .39, yellow catfish .54, and jack salmon .35.

The small mouth bass, the most active fish, shows the greatest difference between aerated serum and venous blood carbon dioxide tensions, *i. e.*, pH units. The carbon dioxide tension differences between aerated serum and venous bloods here indicated by these observations might be more an index of the resistance that the fish exerted against the preparation and the drawing of blood from the conus arteriosus and not the true picture of the blood under normal conditions. Taking all these factors into consideration the pH of the aerated serum is a better index of the pH of arterial blood under normal conditions than any other one factor measured.

The experimental error in the observations, and the fact that venous blood and that usually of a struggling fish was tested, would account for the wide variations in the pH of the venous blood of a given species. It is the opinion of the authors that with more refined technique the variation in the pH of arterial blood of fishes will be found to be far less than indicated by the

[1]Only three sheepshead and two jack salmon were tested. Thus the data on these two species of fishes are not significant.

TABLE I

DATA ARRANGED IN ORDER OF THE pH DIFFERENCE BETWEEN THE WATER AND THE AERATED WATER FROM WHICH EACH FISH WAS TAKEN

O_2 ml. per L.	pH of Water	pH of Aerated Water	Difference pH Water and Aerated Water	pH of Blood	pH of Aerated Serum	Difference pH Blood and Aerated Serum	Per cent Hemoglobin
			CARP				
8.22	8.35	8.45	.10	7.19	7.95	.77	72
8.22	8.35	8.45	.10	7.35	8.28	.93	42
8.22	8.35	8.45	.10	7.56	8.48	.92	47
6.49	8.60	8.50	—.10	7.22	8.24	1.02	35
10.15	8.92	8.81	—.11	7.32	8.35	1.03	...
7.08	8.80	8.67	—.17	7.35	8.26	.91	52
8.64	8.65	8.35	—.30	7.50	8.37	.87	70
			SMALL MOUTH BASS				
5.22	8.62	8.50	—.12	7.15	8.23	1.08	76
5.22	8.62	8.50	—.12	7.18	8.28	1.10	82
5.35	8.58	8.45	—.13	7.30	8.23	.93	80
5.35	8.58	8.45	—.13	7.47	8.56	1.09	70
6.55	8.58	8.45	—.13	7.55	8.43	.88	78
7.09	8.80	8.67	—.17	7.16	8.09	.93	40
7.09	8.80	8.67	—.17	7.18	8.02	.88	65
....	8.97	8.80	—.17	7.32	8.52	1.20	68
....	8.97	8.80	—.17	7.33	8.52	1.19	60
....	8.97	8.80	—.17	7.52	8.55	1.03	60
8.64	8.65	8.36	—.31	6.89	7.80	.91	46
			PERCH				
7.28	8.30	8.48	.18	6.90	7.80	.90	...
7.33	8.41	8.50	.09	7.20	8.14	.94	30
6.99	8.28	8.20	—.08	7.03	7.99	.96	30
6.99	8.28	8.20	—.08	7.18	8.11	.93	30
6.49	8.60	8.50	—.10	7.47	8.35	.88	...
7.13	8.50	8.37	—.13	7.15	7.95	.80	...
7.13	8.50	8.37	—.13	7.00	8.08	1.08	...
7.65	8.83	8.68	—.15	7.25	8.02	.77	34
			ROCK BASS				
8.47	8.00	8.42	.42	7.64	8.50	.86	48
8.47	8.00	8.42	.42	7.68	8.37	.69	50
7.28	8.30	8.48	.18	7.05	7.90	.85	42
7.28	8.30	8.48	.18	7.15	7.72	.57	47
7.33	8.41	8.50	.09	7.18	8.00	.82	49
7.33	8.41	8.50	.09	7.15	8.15	1.00	46
6.35	8.54	8.54	.00	7.35	8.25	.90	46
6.35	8.54	8.54	.00	7.72	8.52	.80	46
6.35	8.54	8.54	.00	7.08	8.30	1.22	50
6.35	8.54	8.54	.00	7.35	8.30	.95	41
6.38	8.55	8.48	—.07	7.32	8.35	1.03	46
7.34	8.62	8.52	—.10	7.50	8.47	.97	45
7.34	8.62	8.52	—.10	7.75	8.45	.70	44
7.34	8.62	8.52	—.10	7.20	8.25	1.05	45
7.34	8.62	8.52	—.10	7.30	8.68	1.38	45
5.22	8.62	8.50	—.12	7.02	7.92	.90	42
7.13	8.50	8.37	—.13	7.08	8.32	1.24	44
7.13	8.50	8.37	—.13	7.08	8.08	1.00	52
5.35	8.58	8.45	—.13	7.18	7.90	.72	50
7.66	8.83	8.68	—.15	7.65	8.44	.79	...
....	8.97	8.80	—.17	7.32	8.47	1.15	...
....	8.85	8.65	—.20	7.22	8.25	1.03	49
....	8.83	8.62	—.21	7.05	7.80	.75	52

data given in this paper. This is borne out by the fact just given that the pH range of aerated serum is less than the

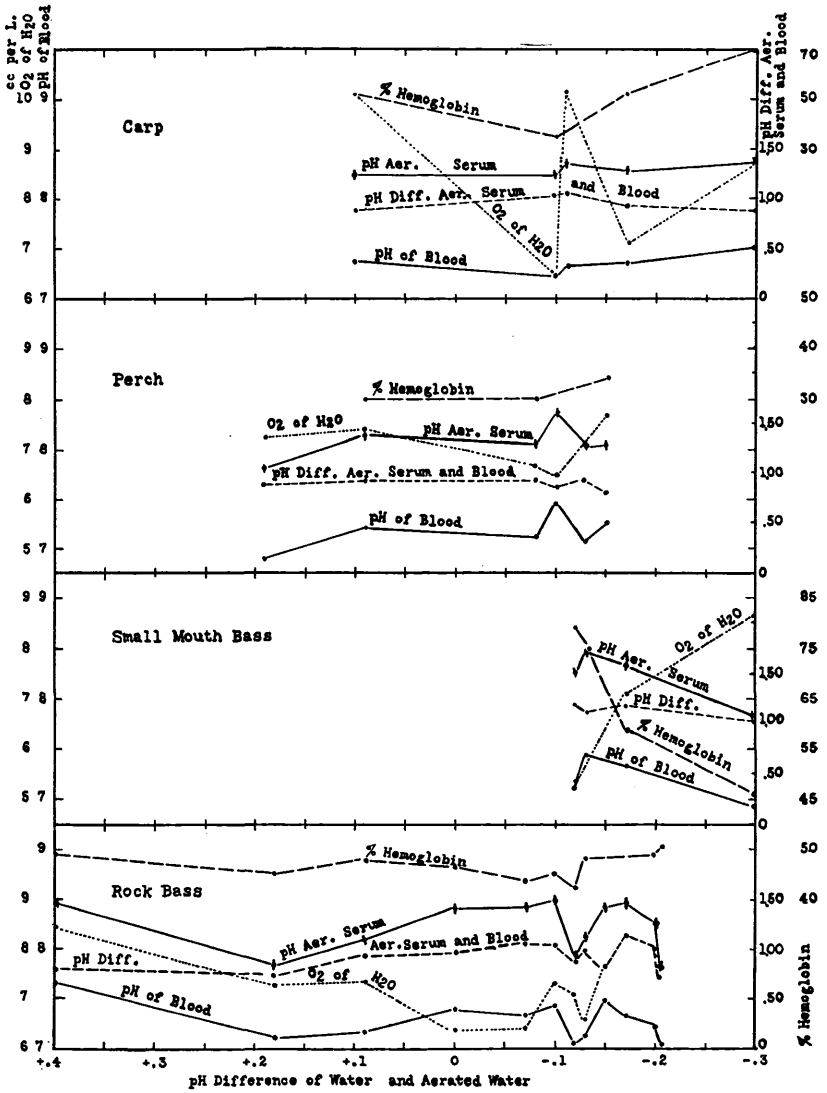

Fig. 1. A graphic representation of the data in Table I.

difference between the pH of the venous blood and aerated serum. By referring to the two tables it is seen that all pH readings of aerated serum are without exception well on the alka-

TABLE II

DATA ARRANGED IN ORDER OF THE pH OF THE BLOOD OF EACH SPECIES OF FISH

pH of Blood	pH of Aerated Serum	Difference pH Blood and Aerated Serum
CARP		
6.90	7.75	.85
6.92	7.76	.84
6.96	7.72	.76
6.97	7.69	.72
7.03	7.83	.80
7.08	8.06	.98
7.19	7.95	.77
7.22	8.24	1.02
7.32	8.35	1.03
7.34	8.19	.84
7.35	8.27	.92
7.50	8.37	.87
7.56	8.48	.92
ROCK BASS		
6.71	7.80	1.09
6.82	7.46	.64
6.85	7.85	1.00
6.89	7.93	1.04
6.92	7.48	.56
7.02	7.92	.90
7.04	7.75	.71
7.05	7.90	.85
7.05	7.80	.75
7.08	8.08	1.00
7.08	8.30	1.22
7.08	8.32	1.24
7.15	8.15	1.00
7.15	7.72	.57
7.18	7.90	.72
7.18	8.00	.82
7.20	8.25	1.05
7.22	8.25	1.03
7.30	8.68	1.38
7.32	8.35	1.03
7.32	8.47	1.15
7.35	8.25	.90
7.35	8.30	.95
7.50	8.47	.97
7.64	8.50	.89
7.65	8.44	.79
7.68	8.37	.69
7.72	8.52	.80
7.75	8.45	.70

pH of Blood	pH of Aerated Serum	Difference pH Blood and Aerated Serum
PERCH		
6.70	7.50	.80
6.76	7.66	.90
6.80	7.74	.94
6.90	7.80	.90
7.00	8.08	1.08
7.03	7.99	.96
7.15	7.95	.80
7.18	8.11	.93
7.20	8.14	.93
7.25	8.02	.77
7.47	8.35	.88
SMALL MOUTH BASS		
6.44	7.56	1.09
6.60	7.80	1.20
6.89	7.80	.91
7.00	7.68	.68
7.15	8.23	1.08
7.16	8.09	.93
7.18	8.40	1.22
7.30	8.23	.93
7.32	8.52	1.20
7.33	8.52	1.19
7.47	8.56	1.09
7.52	8.55	1.03
7.55	8.43	.88
SHEEPSHEAD		
7.16	7.80	.50
7.22	7.82	.36
7.34	7.83	.31
CATFISH		
6.63	7.46	.83
6.72	7.12	.40
7.03	7.49	.46
7.10	7.55	.45
JACK SALMON		
6.87	7.20	.33
6.99	7.36	.37

line side of neutrality. This is in keeping with the findings of the pH of bloods of all species of fishes reported by Root (1931) and Willmer (1934)[2] (calculated) when the blood was equilibrated with a carbon dioxide partial pressure approximating a carbon dioxide tension found in natural waters where fishes live. Their

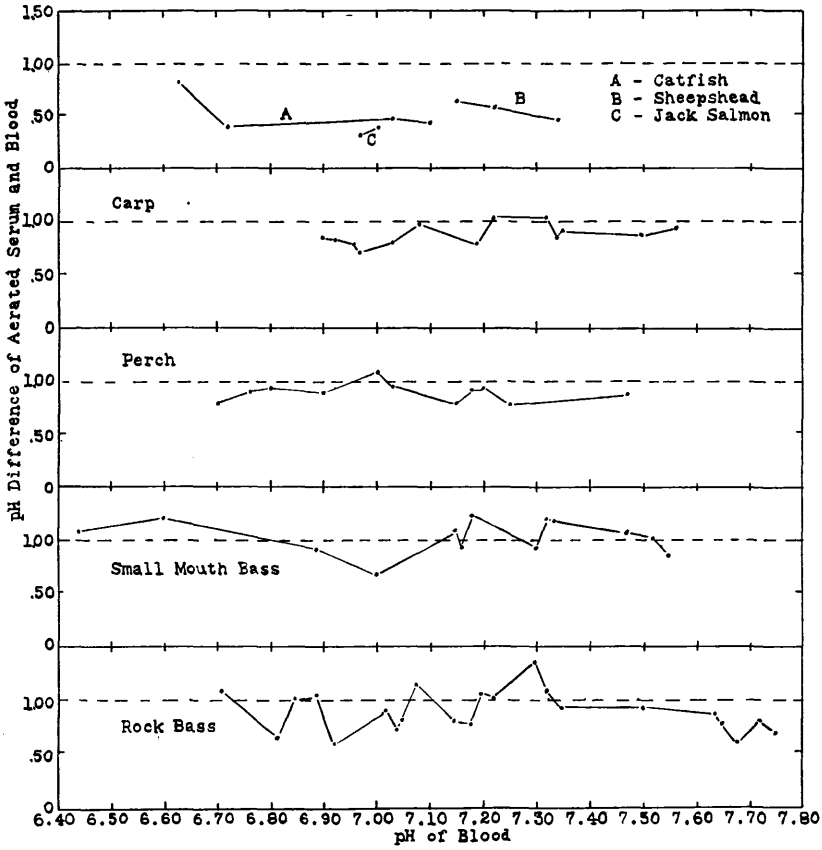

Fig. 2. A graphic representation of the data in Table II.

drawn blood becomes acid only when equilibrated with a carbon dioxide partial pressure in mm. Hg in toad fish of 25.40, in the sea robin of 28.10, in mackerel of 65.05, (Root, 1931), and 25.+ of certain other fishes (Willmer, 1934). These are even higher than the carbon dioxide tension (20.+ mm. Hg) of the experimental water in which fresh water fishes ceased to absorb

[2]The pH given for O mm Hg CO_2 is questionable. The method of obtaining the pH's is not clear.

oxygen efficiently (Powers, Rostorfer, Shipe and Rostorfer, 1938). The blood of any fish would never come in contact with carbon dioxide tensions of as high values as given above under normal conditions except in the rete mirabile and the gas glands of deep water fishes.

When we go back and re-examine the data obtained by Powers, Rostorfer, Shipe and Rostorfer (1938) it is found that the pH of the blood of an experimental fish was almost without exception on the acid side of neutrality. The pH difference between blood and aerated serum showed excellent expected correlation with the carbon dioxide tension of the experimental water. In these experiments just cited neither the pH of the venous nor the pH of the aerated serum represent the true or actual pH of arterial blood. This is true of the aerated serum since the carbon dioxide partial pressure (.35% At.) especially was not the same as the carbon dioxide tension (the carbon dioxide tension of the experimental water) in which the arterial blood was in equilibrium. The fishes were suffering from extreme anoxemia and were near death and were not compensating for the accumulation of lactates or lactic acid. The experiments do show a definite cycle in the adjustments of the blood even under adverse conditions.

There are no data on possible seasonal changes in blood reactions.

Both Root (1931) and Willmer (1934) as well as Powers and Hickman (1932) have shown that when fish blood is exposed to higher carbon dioxide tensions the hemoglobin rapidly loses in capacity to combine with oxygen. This is a physiological mechanism which offers strong evidence in support of the theory of deposition of gases into the swim bladder of fishes with rete mirabile gas gland mechanisms (Powers, 1932). It now only remains to be shown whether or not this loss of combining power of hemoglobin at high carbon dioxide tensions is reversible to determine the efficiency of the mechanism of deposition of gases into the swim bladders of the fish.

HEMOGLOBIN

The hemoglobin content of the blood was determined with the Dare Hemoglobinometer. The data in Table I are the per cents of hemoglobin when 100% represents 16 grams per 100 cc. of blood which is taken as normal for human blood. Here we have only slight variations from the average of a

species and of all species observed. Again this indicates that a fish has a well adjusted blood system.

CONCLUSIONS

From these observations it is concluded that under varied but natural conditions the pH of the blood of a species of fish is fairly constant and that this uniformity of the blood including the hemoglobin content is maintained by mechanisms similar to those of higher animals.

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