V. A COMPARATIVE STUDY OF THE OXYGEN-CARRYING CAPACITY OF THE BLOOD OF WATER- AND AIR-BREATHING TELEOSTS

The oxygen-carrying capacity of the blood of a number of fishes has been determined by a number of authors including Hall & Gray (1929), Root (1931), Redfield (1933), Willmer (1934) and Black (1940). The results obtained are based either on the estimation of the iron present in the blood or the measurement of the oxygen with the help of Van-Slyke's manometric apparatus. The results indicate that active, fast moving fishes possess a higher oxygen-carrying capacity than normal ones of the similar habitats. Mackrel and Bonito for instance, are known to have a very high concentration of haemoglobin, almost equal to that of mammals though the marine fishes have a lesser quantity of haemoglobin than most of the vertebrates. Some of the fresh-water fishes living in swamps containing acidic water are also known to have a higher oxygen-carrying capacity (Black-1940). No investigations have been however conducted to determine the nature of the oxygen-carrying capacity of the air-breathing teleosts as such. A number of air-breathing fishes therefore have been examined to estimate the oxygen-carrying capacity and by way of comparison, water-breathing fishes of similar habitats have also been investigated.

The oxygen-carrying capacity of the blood of the vertebrates primarily depends upon the amount of haemoglobin present in it. A change in the quantity per unit volume of this respiratory pigment also brings about a change in the oxygen-carrying capacity. A comparative study of the haemoglobin content of the blood of the water-breathing fishes with the air-breathing ones living in similar habitats should give an idea of the differences existing if any, in the nature of their capacity to carry oxygen.

Haemoglobin is a complex compound belonging to a group of conjugating proteins. It is formed by the union of four protoheme molecules with a protein globin. The protoheme molecules are the products, resulting from the combination of proto-porphyrin with ferrous iron. The ferrous iron combines with the oxygen at a higher pressure of this gas and releases readily at a lower pressure existing inside the body. It is estimated that for each atom of iron, two atoms of oxygen are absorbed. The iron present in the blood is almost confined to haemoglobin. Hence the amount of iron present in the blood is always taken as proportional to its oxygen-carrying capacity.

<u>The technique employed</u>

The amount of iron present was estimated by the method of Kennedy using the Klett-Summerson Photoelectric Colorimeter.

Principle

"The material is oxidised by wet ashing. The acid solution is made alkaline and boiled to change phyrophosphate to orthophosphate. Thiocyanate is added, the ferric cyanate is extracted with amyl alcohol and determined colorimetrically."

Procedure

Transfer a known quantity (e.g. 1.0 cc.) of the blood to kjeldahl's flask of 100 cc. capacity. Add to it 5.0 cc. of iron free concentrated sulphuric acid and 2.0 cc. of 60 % perchloric acid. Digest the substances by gently heating over a low flame until the solution becomes colourless and dense fumes of SO_3 are given off. Allow the solution to cool. Meanwhile take two more kjeldahl's flasks. Add in one 5.0 cc. of the standard solution and in another double distilled water. Treat these two liquids exactly in the same way and allow them to cool. When cooled, add a drop of concentrated Nitric acid to each of these solutions and dilute them to 100 cc. Label them as unknown, standard and blank respectively.

(The standard iron solution is prepared in this way. Dissolve 0.702 gm. of reagent grade crystalline ferrous ammonium sulphate in 100 cc. of water and add to it 5.0 cc. of iron free conc. free sulphuric acid. Warm the solution slightly and add potassium permanganate drop by drop until one drop produces permanent colour. Pour this solution in one litre volumetric flask with rinsings. Dilute the solution to the mark and mix thoroughly. The solution contains 0.1 mg. of ferric iron per cc.)

Transfer 10 cc. of each of these solutions in separate 50 cc. glass stopered cylinders. To each add 10 cc. of Amyl alcohol and 5.0 cc. of potassium thiocyanate and shake them thoroughly. By using a green filter in this process first set the colorimeter to zero density with amyl alcohol solution which settles on the blank and then estimate the densities of the Amyl alcohol solutions settled on the standard and unknown respectively.

The specimens of the fishes were collected alive and carried to the laboratory in suitable containers provided by the Department of Fisheries, Bombay. As soon as the fresh-water fishes were brought to the laboratory, they were transferred to the aquarium. They were removed from water only just before dissecting them for extracting the blood. The marine fishes were kept in suitable containers containing marine water which was replaced periodically. Only the venous blood extracted from the ventricle with the aid of a suitable syringe previously washed with 5 % neutral potassium oxalate solution was used for analysis. The test-tubes in which the blood was collected were the ones on which a thin uniform film of potassium oxalate solution was allowed to settle along their inner surfaces. After transferring the blood to these test-tubes they were gently rotated to enable a sufficient quantity of oxalate to dissolve and prevent the clotting of the blood. The tubes then were stoppered immediately to prevent evaporation. The necessary tests were carried soon after, the blood being shaken once again thoroughly but gently to ensure uniform distribution of the corpuscles in the blood.

Results

Atleast 6 readings were recorded for each fish.

The amount of iron determined in mg. per 100 cc. of the blood in the fishes investigated is as follows :-

Name of the fish	The amount of iron estimated	
	Minimum	Maximum
Water-breathing fishes		
1. *Glyphidodon caelestinus* (Cuv. & Val.)	17.80	26.87
2. *Labeo rohita* (Ham.)	15.13	19.83
3. *Catla catla*	24.46	27.52
4. *Cirrhina mrigala* (Ham.)	18.38	24.31
5. *Tetrodon patoca* (Ham.)	20.17	23.38
Air-breathing fishes		
6. *Macrones gulio* (Ham.)	31.42	35.38
7. *Heteropneusteus fossilis* (Bloch)	29.41	36.23

Name of the fish	The amount of iron estimated	
	Minimum	Maximum
8. *Boleopthalmus dussumieri* (Cuv. & Val.)	30.40	37.60
9. *Osphronemus gourami* (Lacep)	32.17	36.96
10. *Ophiocephalus striatus* (Bloch)	32.87	40.00

N.B. :- The specimens of fishes collected for this piece of investigation comprise both fresh-water and marine teleosts. The water-breathing *Tetrodon* and the air-breathing *Macrones* and *Boleopthalmus* were collected from Mahim creek. The water-breathing marine *Glyphidodon* was collected from the sea-cost of Chowpatty, Bombay. All the fresh-water water-breathing fishes as well as the air-breathing *Osphronemus* and some specimens of *Ophiocephalus* were collected from the Bandra tank. Other specimens of *Ophiocephalus* were collected from the river Sabarmati at Ahmedabad, while the specimens of *Heteropneusteus* were collected from a tank situated in the Aarey Milk Colony, Goregaon, Bombay.

Discussion

The haemoglobin concentration of blood based on iron estimation has been estimated by a number of workers including Hall & Gray (1929). The results obtained by Hall & Gray (1929) are as follows :-

Name of the fish	The amount of iron estimated in mg. in 100 cc. of the blood	
	Minimum	Maximum
Water-breathing fishes		
1. Rudder fish	17.3	25.0
2. Puffer fish	17.1	27.8
3. Toad fish	12.5	15.2
4. Bonito	37.0	52.9
5. Mackrel	34.3	51.0

The first three of the above fishes are non-migratory while the last two are well-known for their migratory habits.

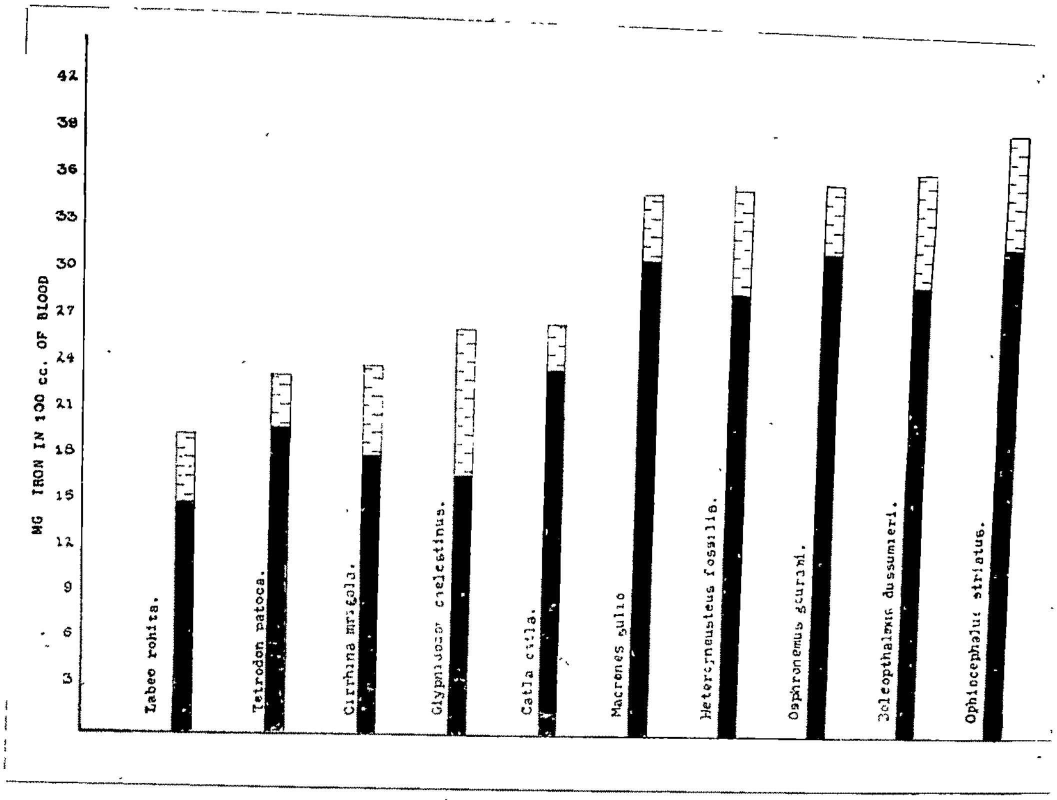

Fig.66. Histogram showing the iron content in mg. per 100 cc. of the blood in the fishes investigated.

Root (1931), Redfield (1933), Willmer (1934) and Black (1940) have estimated the oxygen-carrying capacity of the blood by determining oxygen present per 100 cc. of the blood. Some of the results obtained by them in certain teleostean fishes are as follows :

Name of the fish	Mean oxygen-carrying capacity cc.oxygen/100 cc.blood
Water-breathing fishes	
1. Goose fish	5.10
2. Puffer fish	6.80
3. Toad fish	6.20
4. Paku (Myleus setiger)	10.78
5. Haimara (Hoplias malabaricus)	6.53
6. Common sucker (Catostomus)	10.60
7. Carp (Cyprinus carpio)	12.50
8. Cat fish (Ameiurus nebulosus)	13.30
9. Mackrel	15.80
Air-breathing fishes	
10. Eel (Anguilla japonica)	12.90
11. Eel (Electrophorus electricus)	19.70
12. Hossa (Hoplosternum littorale)	18.14

The results obtained by Hall & Gray (1929), as seen from their investigations based on the estimation of iron, show that migratory fishes have a higher oxygen-loading tension of blood than the non-migrating water-breathing fishes. Their investigations show that the amount of iron present in the blood of non-migratory fishes varied from a minimum of 12.5 mg. in toad fish to a maximum of 27.8 mg. in the puffer fish. The corresponding figures for migratory fishes vary from a minimum of 34.3 mg. in Mackrel to a maximum of 52.9 mg. in Bonito.

The investigations conducted by Redfield (1933), Willmer (1934) and Black (1940) also confirm the findings obtained by Hall & Gray. Those of the former investigators also reveal that oxygen-leading tension is higher in the active fish of the open river viz. Myleus setiger, the cat-fish inhabiting swampy acidic waters viz. Ameiurus, a sucker-fish Catostomus, a non-migratory carp Cyprinus carpio, as well as air-breathing fishes Anguilla japonica, and Electrophorus electricus and Hoplosternum littorale.

The present investigations show that the haemoglobin concentration of the blood based on the estimation of iron is different for water-breathing and air-breathing fishes. For water-breathing teleosts, the amount of iron varies from a minimum of 15.13 mg. in Labeo rohita to a maximum of 27.52 mg. in Catla catla. The corresponding figures for air-breathing fishes vary from a minimum of 29.41 mg. in Heteropneusteus to a maximum of 40.0 mg. in Ophiocephalus.

Conclusion

The conclusion that could be drawn from this piece of investigation is that the oxygen-loading tension of the blood depends upon a number of factors. Under the normal conditions this oxygen-loading tension in the air-breathing fishes is higher than that in the water-breathers. Of the fishes living under normal conditions, only a single case of Cyprinus carpio is known to have a higher figure.