SOME OBSERVATIONS ON THE RESPIRATION OF CERTAIN TROPICAL FRESH-WATER FISHES

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(With Sixteen Text-figures.)

INTRODUCTION.

CARTER AND BEADLE (1931), as the result of their work on the fauna of the swamps of the Paraguayan Chaco, pointed out that the fishes and other animals of such localities are living under very special respiratory conditions. In many of the swamps which they investigated, the oxygen content of the water was found to be extremely low, so low in fact as to make aquatic respiration, as ordinarily understood, inadequate for the needs of the fish, unless the blood had become strangely modified to function at very low oxygen tensions. The work of Krogh and Leitch (1919) established that such a modification had apparently taken place in the blood of the carp, pike and eel, and was therefore presumably possible in the case of the fish living in these swamps. These authors showed that the blood of the fish mentioned was saturated at much lower oxygen tensions than was the blood of the cod or plaice. Many of the fish which Carter and Beadle found in the Chaco had adopted air-breathing habits, undoubtedly as an adaptation to the conditions. For certain reasons Carter and Beadle suggested that the gills still remained functional as organs of excretion of CO₂, and were more efficient in this direction than the newly acquired respiratory organs, which were primarily concerned with oxygen intake. Such organs were found in several situations in the body of the fish, such as on the surface of the gill chamber, the stomach, the intestine and in some cases the air bladder. The evidence therefore suggested that aquatic respiration had for some reason or other been discarded in favour of aerial respiration, and that the adaptation of the blood to low oxygen tensions such as is found in the carp, pike and eel, had either not occurred or had proved inadequate. Possibly other factors besides oxygen tension entered into the situation.

Altogether the conditions reigning in more or less stagnant tropical waters are so peculiar that an expedition was fitted up to investigate the waters of the rivers and swamps in British Guiana, and to examine the behaviour of the fish in their different surroundings. The Forestry Department of the Government of British Guiana very kindly provided adequate laboratory accommodation of a primitive type and

1 The author wishes to express his indebtedness to the various bodies which made grants towards the cost of the expedition, and especially to the Trustees of the Percy Sladen Memorial Fund and the Government Grant Committee of the Royal Society. He also wishes to express his thanks to Dr. F. J. W. Roughton for helpful criticism.
every available facility for carrying out such an investigation. The laboratory consisted of rooms in the hospital of the now abandoned penal settlement on the bank of the Mazaruni River. Indian fishermen were employed to obtain fish from the main river, which was, at Mazaruni Settlement, something over a mile wide; from the “creeks” or streams, which were often only a few yards in width, except during the floods in the wet season; and from the more stagnant swamps and ponds which were only occasionally or never connected with the creeks. It was perhaps unfortunate that the period of the investigation coincided with the long wet season in British Guiana, when owing to the enormous rise in the level of the rivers, fish are not very plentiful, being presumably scattered far and wide over the countryside, whereas during the dry season they are more concentrated in the main rivers, and often tend to get trapped in holes as the waters recede.

From the present point of view, the waters investigated may be placed in three groups: (a) river water, (b) creek water, and (c) waters of the swamps and ponds. The oxygen contents of these waters were found by Carter\(^1\) to fall within the following limits, at temperatures of 25–30\(^\circ\) C., which was found to be the usual range for these waters:

(a) River 4.5–5.0 c.c. per litre.
(b) Creek 2.5–3.5 c.c. per litre.
(c) Swamps 0.2–2.0 c.c. per litre.

These figures correspond to the following tensions in mm. Hg:

(a) River 120–135 mm. Hg.
(b) Creek 65–95 mm. Hg.
(c) Swamp 5–55 mm. Hg.

It is at once evident that occasions must frequently occur in the swamps and ponds when blood would leave the gills of the fish in an unsaturated condition unless its haemoglobin has a greater affinity for oxygen than normal, as has that of the blood of the carp, pike and eel. Even then the extreme conditions in the swamp would test it very severely. It was not surprising therefore to find that most of the fish in the swamps had adopted aerial respiration, or lived entirely in the surface layer, where the water was oxygenated by direct contact with the air.

Besides having a very low oxygen content these waters were found to contain a considerable quantity of CO\(_2\) and, in addition, they were very acid in reaction. This was particularly true of the swamp water, and the acidity was found to be due to other acids besides CO\(_2\). Carter records the following CO\(_2\) tensions:

(a) River 2–5 mm. Hg (may rise to 18 at the height of the wet season).
(b) Creek 6–22 mm. Hg.
(c) Swamp 16–32 mm. Hg.

Now CO\(_2\) has been shown by Krogh and Leitch (1919) and by Root (1931) to have a considerable effect in liberating O\(_2\) from combination with haemoglobin in the

1 The chemical nature of these waters will be discussed in a paper by Dr G. S. Carter, giving the results of observations made by him at the same time as those given in this paper.
blood of marine fishes, and if this were found to hold good for the fishes of these creeks and swamps their haemoglobin would be rendered practically useless.

Certain fish were therefore selected by reason of their size and abundance from the three different situations, rivers, creeks and swamps, and their blood was investigated with regard to its oxygen capacity and its saturation under various CO₂ and O₂ pressures.

From the river were selected the paku (*Myleus setiger* Mull and Troschel), the baiara (*Hydrolycus scomberoides* (Cuv.)) and the bom-bom (*Pterodoras granulosus* (Val.)). Certain other fish which were brought in by the fishermen were also examined. From the creeks the haimara (the adult of *Hoplias malabaricus* (Bloch.)) and the electric eel (*Electrophorus electricus* (L.)) were chosen, and from the swamps and trenches the hassa (*Hoplosternum littorale* Hancock). The paku is a fish which is always obtained in the open river, and the favourite place in which the Indians catch it is in the rapids and falls. The baiara also frequents the open river. The bom-bom, although a very characteristic and common fish in the river, is essentially a bottom feeder and mud-lover. Of the creek fish the haimara is typical of the fast-flowing highly oxygenated creeks, while the electric eel is found in those which are more stagnant and muddy. The hassa can live almost anywhere but it especially frequents the trenches, swamps, and ponds which have very little oxygen. Another fish, the yarrow (*Erythrinus erythrinus* (Schn.) or *Hoplerythrinus unitaeniatus* (Spix.)) is characteristic of the more stagnant waters, and its behaviour will be discussed in some detail. Of the above fish the electric eel, the hassa, and the yarrow are all known to breathe air, the eel by a modification of the pharyngeal cavities, the yarrow by its air bladder, and the hassa by a modification of the intestinal wall. Among the fish selected therefore a series is formed from the paku which is only found in highly oxygenated creeks to the yarrow, which is characteristic of the stagnant swamps. In an investigation of the blood of fish the choice is necessarily limited by the size of the fish and the amount of blood available from it. With the exception of the hassa and the yarrow the fish chosen were capable of yielding over 10 c.c. each. For the investigation of the blood of the hassa about fifteen specimens were taken and about 5 c.c. of blood obtained. The yarrow could not be caught in sufficient numbers to obtain blood in quantity, and consequently its respiration was investigated by different means.

**EXPERIMENTAL METHODS.**

Since the expedition's camp was likely to be mobile, in order to sample the various classes of water, only very portable apparatus was taken out and perhaps rather unorthodox methods were employed. The immediate object was to obtain oxygen dissociation curves for the various bloods, first in the absence of CO₂ and then under known CO₂ pressures, actually 10 and 25 mm. Hg. For this purpose

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1 The identification of these fish has been carried out by Dr J. R. Norman of the British Museum (Natural History) to whom the author wishes to express his thanks.

2 The experiments on this fish were mostly carried out on one individual, the identity of which is slightly uncertain, as there appear to be two closely related genera, which at the time of the experiments were not distinguished. It is probable that the behaviour of both is identical as confirmatory experiments were performed on other individuals with precisely similar results.
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1 c.c. of blood was placed in a 50 c.c. tonometer of the type described by Barcroft (1933), which was then evacuated by the repeated lowering of a mercury levelling bulb, and releasing the air thus drawn from the bulb by means of a two-way tap. The blood was then slowly shaken in vacuo for 10 min. so that all O₂ and CO₂ were drawn off. Air was then readmitted in sufficient amount to give the desired oxygen pressure, which was obtained by observing the level of a mercury manometer attached to the evacuating bulb. The tonometer was then rotated backwards and forwards at frequent intervals for half an hour, during which time it remained connected with the manometer so that the O₂ pressure could be controlled. Owing to the extreme constancy of the laboratory temperature, a water bath was dispensed with except for one or two particular experiments. Nearly all the readings were made with an air temperature of 28° C., and the extreme limits were 26 and 30° C. After equilibration the blood was drawn into the pipette by slightly lowering the pressure in the evacuating bulb, which was attached to the tonometer by means of the pipette. The blood was then analysed in a Van Slyke apparatus. 0.2 c.c. samples were analysed, and the tonometer pipettes were graduated to give two such samples. The analyses were carried out according to the standard technique described by van Slyke and his co-workers (1924, 1927). The method of equilibration adopted is perhaps not as accurate as might be desired, but it had two very important advantages for the particular occasion. The apparatus was reasonably portable, and could be worked single-handed sufficiently rapidly to obtain several points on a dissociation curve, or even a complete curve, in one day; the latter being rather an important consideration when working at temperatures over 80° F. with only a limited supply of ice available. During the time the first sample in the pipette was being analysed the second sample was kept cool, and by this means it was found that much more concordant readings were obtained, since the oxygen consumption by the blood was to some extent reduced by the cooling.

Blood was obtained from the fish either by direct bleeding from the cut gill vessels, or by dissection of the heart and withdrawal of the blood from the sinus venosus by the use of a hypodermic syringe. The latter was in most cases found to be the more satisfactory method. As soon as the blood was drawn it was whipped with a feather, and then filtered through muslin. Some of the bloods clotted with extreme readiness so that the whipping could hardly be started in time, in others the separation of fibrin was long delayed in spite of vigorous whipping. On mixing the ferricyanide with the blood in the Van Slyke apparatus a further coagulum was produced, which rendered it necessary to shake the blood for at least 4 instead of the usual 3 min. before equilibrium was obtained. The clot tended to stick to the sides of the vessel but was easily removed later by means of weak caustic soda.

When dissociation curves were being obtained under different pressures of CO₂, the blood in the tonometer was evacuated in the same way, and air admitted to the required O₂ pressure. The CO₂ was then admitted from a reservoir till the pressure recorded by the manometer had fallen by 10 or 25 mm. as the case might be. The CO₂ in the reservoir was periodically analysed in the Van Slyke apparatus to check the amount of CO₂ present. The CO₂ was obtained from cylinders. After the gases
had been admitted to the tonometer they were thoroughly mixed by raising and lowering the mercury in the evacuating bulb which remained connected with the tonometer throughout the equilibration. This was found to be very necessary, since otherwise the CO₂ tended to remain in excess in the tonometer into which it was drawn, and which was only connected with the evacuating bulb by the long narrow pipette. The total volume of the tonometer and evacuating bulb was in the neighbourhood of 300 c.c., so that the gases absorbed by the blood caused only a negligible alteration in the pressures.

EXPERIMENTAL RESULTS.

The first fish whose blood was examined was the paku, and the dissociation curves obtained are shown in Fig. 1. There is nothing unusual in the curve for zero CO₂ pressure, and it is very similar to the dissociation curve for the blood of marine fish and for many other known bloods at the temperatures at which they are normally working. The other two curves, although slightly higher, also agree well with those found by Root for marine fish, and it is obvious that the CO₂ has a profound effect in displacing the oxygen from combination with the haemoglobin. If the blood in the gills comes into equilibrium with the outside water, the range of the paku is very clearly limited by the CO₂ content of the water in which the fish is living; a fact which probably explains the predilection of this fish for rapidly flowing, or much disturbed water such as is found near the falls. Under the conditions prevailing in the creeks the blood would only be about 80 per cent. saturated, more in rapidly flowing creeks, but much less in the sluggish ones. In the swamps the blood could never be more than, say, 40 per cent. saturated. There is nothing particular therefore

![](image-url)
to note about the paku; it conforms to the marine type of fish and demands water with high O₂ and low CO₂ content—especially the latter.

The baiara (Fig. 2) behaves very similarly to the paku, except that CO₂ appears to have rather less effect on the O₂ content. For example, choosing the point for which actual data were obtained, at 25 mm. CO₂ pressure and 40 mm. of oxygen the paku blood is only 43 per cent. saturated while the baiara is 63 per cent. saturated. The blood of both fish, however, is very adversely affected as an O₂ carrier if CO₂ is allowed to come in contact with it.

The haimara is an interesting fish. It is a fish which is normally caught in creeks, and only to a lesser extent in the open river, but the creeks in which it is obtained are usually rapidly flowing and have either a sandy or rock bottom and their water is well oxygenated. The dissociation curves for the haimara are shown in Fig. 3 and they are seen to be similar to those for the paku, and this fact would seem to limit the range of the haimara and prevent its entering stagnant waters, unless it has some other means of oxygenating its tissues.

From the information which could be obtained from the Indians it would appear that the adult haimara is generally confined to the rapid creeks and smaller rivers, although they also say that in the dry season haimara are frequently found in pools which have been formed in the bed of the creek as the latter dried up. Unfortunately there was no opportunity for studying the O₂ and CO₂ content of the water of such pools, but from the amount of suspended organic matter in the river it would be reasonable to expect that in the stagnant pool the CO₂ content would be high, so it remains rather a puzzle as to how the haimara accommodates itself under these conditions.

![Fig. 2 Oxygen dissociation curves for baiara blood at zero and 25 mm. Hg CO₂ pressures](image-url)
When the blood of the bom-bom, hassa and electric eel was examined (Figs. 4, 5 and 6) a very different state of affairs was found. The blood of these three fish was comparatively little affected by the presence of CO₂. In the case of the electric eel the effect of 25 mm Hg CO₂ was practically within the limits of the experimental error of the method. The advantage gained by the fish is obviously very great. Their
range can be extended into nearly stagnant creeks and into those pools, characteristic of the tropics, where decay is proceeding very rapidly in the vegetation and fallen leaves in the water, and the CO₂ content is high. In fact, from the point of view of respiration the extent of their range becomes practically dependent on the oxygen content of the water, and many of the fish overcome this difficulty by acquiring subsidiary means for obtaining oxygen. The gills of most of the air-breathing fish examined remain functional, although in the case of the hassa they are reduced and never capable of supplying all the respiratory needs of the fish. It is possible therefore that in these fish the main function of the gills has somewhat changed from one of obtaining oxygen to one of excreting CO₂.

![Fig 5. Oxygen dissociation curves for hassa blood at zero and 25 mm. CO₂ pressures](image)

It will be noticed from the dissociation curves shown in the diagrams that in the absence of CO₂ there is very little difference in the slope and form of the curves, and they can be nearly superimposed on the normal dissociation curves of bloods of widely different types of animal. If the usual temperature effects on the haemoglobin hold good for the blood of these fish, their blood would probably become slightly more easily saturated than that of the marine fish investigated by Root (1931), but under the conditions in which the animals normally live there is no modification of the blood in this direction, which perhaps shows that over a wide range of animals the tissues make very similar demands on the oxygen of the blood, and that although animals live under diverse conditions the normal dissociation curve of the blood is maintained the same.

Before discussing any further the reasons and possible causes of the difference in behaviour of the blood of the electric eel, etc., and that of the paku for example, it will be profitable to record the results of experiments of a somewhat different
nature on the yarrow. As already mentioned the yarrow is a fish of relatively stagnant waters and is an air-breather. It does not always breathe air, and can, under certain conditions, remain below the surface for very long periods during which its gills are functioning and supplying all its respiratory needs. On other occasions it visits the surface at regular intervals, generally every three or four minutes, and its gill operculum remains closed over the gill chamber. The fish therefore can be used as a very good indicator of the respiratory efficiency of the surrounding water, and by studying the conditions of the water in relation to the behaviour of the fish much can be learnt about the control of respiration in the fish.

After a series of preliminary experiments of a more or less unsuccessful nature it became clear that there were at least three factors which chiefly influenced the behaviour of the fish. These were the hydrogen-ion concentration, the CO₂ content and the oxygen content of the water. The first experiments were conducted in river water but no reason could be detected for the behaviour of the fish, and the experiment seemed hopeless. The fish was then kept in a large glass jar containing water from the pond in which it had been caught, and by means of two tubes leading down to the bottom of the jar the water could be oxygenated or made to contain more or less CO₂ at will. In its natural water there seemed to be some correlation between the O₂ and CO₂ content of the water and the type of respiration. This last was divided into three categories: aerial respiration, when the fish was regularly taking bubbles of air and the gill operculum was closed, intermediate respiration when it made frequent visits to the surface but its gills remained functional, and finally aquatic respiration when it remained permanently, that is, for more than ten minutes, below the surface and its gills were obviously satisfying its needs. A few

Fig. 6. Oxygen dissociation curves for blood of electric eel at zero and 25 mm. Hg CO₂ pressures.
observations were also made on the rate and extent of the movements of the gill operculum, both of which show considerable fluctuation and would in themselves prove an interesting study.

Now the most striking difference between the river water and the water of the pond in which the yarrow were living was found to be one of hydrogen-ion concentration. River water is in the neighbourhood of pH 6.7, while the pond water ranged from pH 3.8 to 5 or thereabouts. Consequently in the experiment on the yarrow it was necessary first of all to keep the water between those limits. Actually observations were only made when the pH of the water lay between 4 and 5. The pH was determined colorimetrically by means of indicators, and is subject to a certain amount of error owing to the deterioration of some of the standards under the hot climatic conditions. However, the error is probably never more than ±0.2, and it is evident from the accompanying graph (Fig. 7) that the determination of the hydrogen-ion concentration was sufficiently accurate. On the graph the circles represent the conditions in which aquatic respiration is effective, dots, intermediate respiration, and crosses, aerial respiration. The ordinates represent the CO₂ content of the water, the abscissae the oxygen content. These were both determined by

Fig. 7. Relationship between the type of respiration of the yarrow and the CO₂ and O₂ contents of the water.

© Aquatic respiration. • Intermediate respiration + Aerial respiration.
Respiration of certain Tropical Fresh-water Fishes

extraction of 6 c.c. samples of the water in the Van Slyke apparatus, and subsequent absorption of the gases evolved by caustic soda, and sodium hydrosulphite. The method is obviously not extremely accurate, but again the graph indicates that it was sufficient, and it had the enormous advantage that by its use it was possible to determine the \( O_2 \), \( CO_2 \) and \( pH \) of the water at least four times in an hour, and at the same time observe the behaviour of the fish. Care was always taken that the fish was properly adjusted to its surroundings, so that except where a series of consecutive readings was being taken at least twenty minutes was allowed for the fish to become properly adjusted to the altered conditions.

The graph shows quite clearly that both oxygen and \( CO_2 \) are important in determining the behaviour of the fish, and of the two \( CO_2 \) is the more frequent controlling agent. As long as the oxygen in the water is above 1.5 c.c. per litre the fish need never come to the surface, unless the \( CO_2 \) content of the water rises above about 25 or 30 c.c. per litre, or falls below 10 c.c. per litre. Swamp water, and the waters of such situations as are occupied by the yarrow often contain considerable amounts of \( CO_2 \) up to 25 or 30 c.c. per litre, so that in the natural environment the fish must often be compelled to come to the surface. At 35 c.c. per litre it is forced to close the gill chamber. The question naturally arises as to why the gill chamber should close. The \( CO_2 \) might be expected to have three main effects. (1) It might make the blood less efficient as an oxygen carrier, as it has been already shown to do in the case of the paku, and to a lesser extent in other fish. (2) It might alter the hydrogen-ion concentration of the blood and so upset the equilibrium of the fish. Of this possibility little can be said since our knowledge of the buffering power of the blood is almost negligible. A few somewhat crude experiments on this subject will be described later in this paper. (3) It might raise the \( CO_2 \) content of the tissues to too high a level, which owing to the ready penetration of the cell wall by \( CO_2 \) might start damaging the tissues. Probably at least two of these factors play their part.

In Figs. 8 and 9 an attempt is made to show the probable effect of the \( CO_2 \) and \( O_2 \) in the water on the degree of saturation of the blood with oxygen. The curves are the same as those in Fig. 7 and indicate the limits of the various types of respiration, the other lines indicate the degree of saturation of the blood under the corresponding \( CO_2 \) and \( O_2 \) tensions. In Fig. 9 it is assumed that the blood behaves like that of the paku, in Fig. 8 like that of the bom-bom; and an examination of the figures indicates that in all probability the latter is the more likely. This would be expected on other grounds since all the fish examined from environments where the \( CO_2 \) is likely to be high are of the bom-bom type. Krogh and Leitch (1919) pointed out in their paper on fish blood that the two important points on an oxygen dissociation curve were the tension of loading and the tension of unloading, or the oxygen tensions necessary for 95 per cent. saturation and 50 per cent. saturation respectively. Barcroft (1925) has shown that in man symptoms of distress appear when the blood cannot be more than about 80 per cent. saturated in the alveoli, or in other words man suffers from oxygen want when his arterial blood is less than say 80 per cent. saturated, so that on Figs. 8 and 9 have been drawn lines indicating the conditions under which the blood may be 50 and 80 per cent. saturated. Now in Fig. 8 the line indicating 80 per cent.
Fig. 8. Relationship between the saturation of the blood of the yarrow and the O₂ and CO₂ content of the water superimposed on the same graph as the relationship between the type of respiration and the gaseous content of the water.

In Fig. 8 it is assumed that the blood is of the same type as that of the bom-bom, in Fig 9 as that of the paku.

Fig 9.
Respiration of certain Tropical Fresh-water Fishes

saturation of the blood runs parallel to and almost coincident with part of the curve indicating the limits of aquatic respiration. At any point to the left of the 80 per cent. line the conditions in the water are not adequate to saturate the blood to a workable extent, and consequently the fish is compelled to go to the surface to saturate its blood. The blood coming to the gills however is at least partly venous and is therefore not going to lose oxygen to the water, but will probably lose much of its CO₂. If, however, the oxygen content of the water drops, the fish closes its gills and depends entirely on aerial respiration, and the curve showing the limits necessary for this behaviour is almost coincident with the 50 per cent. saturation curve of the blood (Krogh and Leitch's tension of unloading). To the left of this line, oxygen is presumably lost to the water, and since the blood from the gills supplies many tissues of the fish before being oxygenated in the air bladder this is probably a serious matter for the fish, and hence the closure of the gills. The paku type of blood would, on the above lines of reasoning, be quite inadequate since the blood would seldom be 80 per cent. saturated, and the partly venous blood in the gills would be giving up oxygen during much of the time that intermediate respiration is possible and the gills are open. It is probable, therefore, although it was impossible to test the matter directly, that the blood of the yarrow conforms to the type shown by the bom-bom and electric eel, and then the left-hand limits of the curves in Fig. 8 become directly intelligible.

What then sets the upper limits to the curve, since these do not continue to follow the blood saturation lines? Another factor must come in here, and as suggested above this might be either alteration of the hydrogen-ion concentration of the blood or excess CO₂ in the tissues, and these are essentially different although some of their effects may be indistinguishable. To try to obtain some idea as to which of these two was the important factor experiments were made in which the hydrogen-ion concentration of the water was altered between wide limits. It is at once admitted that the answer from these experiments is not necessarily the answer to the above question, and is only suggestive, since there is the question of the permeability of the gill membranes, which are known to be extremely impermeable to most inorganic ions. However, the results are not without interest and are shown in Fig. 10. There was no question of oxygen lack in these experiments since the oxygen was always kept above 1.5 c.c. per litre. By the judicious addition of lactic acid, sulphuric acid, or tartaric acid to the water, high concentrations of hydrogen ions were obtained, and similarly low concentrations by the addition of NaOH. The water in the experimental jar was frequently renewed so that the substances added should not accumulate and alter the osmotic pressure or salt concentration of the water to any serious extent. For the alkaline readings the fish was sometimes left alone in the water since it was found that, presumably due to the excretions of the fish, the water steadily became more alkaline.

The ordinates show the CO₂ concentrations in c.c. per litre and the abscissae the hydrogen-ion concentrations. The circles, dots, and crosses indicate the types of respiration as before. If the CO₂ concentration is in the neighbourhood of 20 c.c. per litre, the fish appears to be almost independent of pH, and it is only when the
water is getting very acid or alkaline that the fish takes notice of hydrogen-ion concentration. This, however, is only partially true. The fish is slowly susceptible to alkalinity. All the readings to the right of the perpendicular drawn at pH 6·2 were taken within say 30 min. of the time that the pH was brought up from some value between 4 and 5. It was found that prolonged sojourn in water above pH 6 seemed to have a deleterious effect on the fish, which was even more marked when the CO₂ concentration was very much reduced. After such treatment the behaviour became quite erratic and unpredictable. The fish often swam backwards and jumped about in the jar. Presumably the effect is produced by increased alkalinity of the blood with consequent removal of the CO₂. It was observed in the earlier diagrams that lack of CO₂ is a very important factor in causing the fish to come to the surface to breathe air, and it seems reasonable to conclude that the "respiratory centre" of

![Graph showing relationship between pH and CO₂ content.](image)

Fig. 10 Relationship between the pH of the water, its CO₂ content and the type of respiration adopted by the yarrow.

this fish is controlled by the CO₂ content of the blood, which must neither be too high nor too low. Low CO₂ in the blood could be obtained either by low CO₂ in the water or by alkali penetrating into the blood. With regard to the lower limit of CO₂ for aquatic respiration it is interesting to note that in *Erythrinus unitaeniatus* the CO₂ content of the air bladder, which is the accessory respiratory organ of these fish, was found by Carter and Beadle (1931) to correspond to a tension of rather less than 10 mm. Hg, which is of the same order as the tension of CO₂ which causes this fish to come to the surface. At 5 mm. it closes its gills. This suggests that when the CO₂ falls to a certain limit in the outside water the fish can conserve its CO₂ better by aerial respiration, and the latter becomes a necessity when the loss of CO₂ to the water is increased by lowering the CO₂ tension in the water below about 5 mm. An attempt is made to summarise the situation in Fig. 11.
These experiments do not tell us how the centres in the brain which control the type of respiration and the gill movements are put into action. All that they indicate is that ecologically the distribution of the fish and its respiratory behaviour are controlled more by CO₂ than by hydrogen-ion concentration. It is known that CO₂ as such penetrates cells more easily than when dissolved and ionised. But what is not certain in this case is which are the cells which have to be entered. If the gill membrane kept out CO₂, which it certainly does not, then there would be no possibility of such control as seen above; but when the CO₂ reaches the blood does it remain as such and affect the respiratory centre directly or does it ionise and produce its effects by alteration of the hydrogen-ion concentration? The gill membrane is only slowly permeable to acids and alkalis, but the above experiments show that alkali deranges the respiratory control rather more than acid does, which would seem to suggest that the CO₂ was the effective agent and was being removed by the alkali. It was not possible by the apparatus available to determine the pH of the blood of the yarrow, but by dialysing the blood of other fish and estimating the pH of the dialysate colorimetrically (Dale and Evans, 1920) the hydrogen-ion concentration of several other types was obtained. They are shown in the accompanying table (Fig. 12). The yakatu (*Prochilodus rubrotamaeitus* Schromb.) and the pirai (*Serrasalmus rhombeus* L.) are two fish of the open river similar in habits to the baiara. The most striking thing about all these bloods is that they are acid, but it is also obvious that they are in most cases not as acid as the surrounding medium. Unfortunately these measurements were only made towards the end of our stay in British Guiana, and only very few observations could be made, and certain fish such as the paku were at that time unobtainable owing to the state of the rivers. However, it seems fairly safe to draw the conclusion that the gill membranes do not readily

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**Fig. 11.** Diagram illustrating the suggested explanation of the curves in Fig. 7.
allow the passage of acid across them, so that one may assume that CO₂ passes the
membrane freely and in the unionised state. In these acid bloods the CO₂ will tend
to remain unionised, but it is also interesting to note that in all the fish examined,
and they form a fairly representative group, the blood contained the enzyme
carbonic anhydrase which is, in many animals, the agent responsible for the rapid
conversion of H⁺ + HCO₃⁻ into CO₂ and water. Both the acidity and the enzyme
will therefore assist the easy passage of CO₂ across the membranes.

There was however one experiment which may contradict the foregoing argu-
ment, although there is another possible explanation for the anomaly. A yarrow was
cased to close its gills and depend entirely on aerial respiration by reducing the CO₂
content of the water to about 5 c.c. per litre. The O₂ content was above 4 c.c. per
litre. After the fish had become settled in this type of respiration but before it was
seriously affected by the low CO₂ in its blood, a few drops of lactic acid were added
to the medium so as to lower its pH from, say, 4.5 to 4.0. This addition of lactic
acid was invariably followed after one or two minutes by the opening of the gills and
a brief period of aquatic respiration. Either the lactic acid penetrates the gills and

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<th>pH of oxygenated blood 0 mm Hg CO₂</th>
<th>pH of oxygenated blood 25 mm Hg CO₂</th>
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<td>Hamara</td>
<td>6.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Yakatu</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Pirau</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Biara</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Bom-bom</td>
<td>6.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Hasaa</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Electric eel</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

changes the pH of the blood, or liberates CO₂ in the blood so as to alter the respira-
tion, or it liberates CO₂ in the surrounding water which then affects the respiratory
centres of the fish, after penetrating the gill membranes. The last seems a very
plausible explanation which moreover agrees with the argument outlined above,
whereas the hypothesis that the lactic acid penetrates the gill membranes is opposed
to the experiments where pH measurements were taken.

Another simple and at the same time illustrative experiment was performed on
the yarrow. It was again forced into aerial respiration by reducing the CO₂ content
of the water to a low level, and then it was stimulated to activity for a short time by
handling it or poking it with rubber tubing. After a very short period of activity it
was found to be breathing actively by means of its gills. The CO₂ produced by the
movement was evidently sufficient to stimulate the respiratory centre.

In the reduction of the CO₂ content of the water, it is interesting to note, the gill
movements become steadily slower and have a smaller amplitude until they die away
altogether. On the other hand increasing the CO₂ above the upper limit for aquatic
respiration causes rapid and full gill movements, which suddenly cease, after one of
the occasions when the fish has been to the surface. When the CO₂ content was too
high the fish was frequently observed to show short periods of gill activity as though testing the CO₂ content of the water. This was occasionally observed, too, when the oxygen content was below 1.5 c.c. per litre, and also when the CO₂ was too low, but then not as frequently. The more usual behaviour in those circumstances was a very slow and slight opening of the gill operculum, and not full respiration for short periods.

Similar experiments to the above were performed on the hassa, which is also an air-breathing fish, but one in which the gills are apparently never adequate to supply its respiratory needs. The hassa comes to the surface about once every 10 min. (as opposed to 3 or 4 for the yarrow) under whatever conditions it is placed. It closes its gills at a rather higher CO₂ content than the yarrow, and is also forced to close them by low CO₂ tensions.

Finally, a few experiments were made to determine the relation between the pH of the blood of the various fish and its CO₂ content. The experiments were admittedly rather crude, since the colorimetric method for the pH of blood is not particularly accurate, especially under the conditions of these experiments. However, they serve to indicate the general behaviour of the blood. The CO₂ content of oxygenated blood under known CO₂ pressures was estimated in the van Slyke apparatus, and another sample was equilibrated in exactly the same way and its pH determined. The experiments were done on the haimara and the bom-bom. They would have been more instructive if performed on the paku and electric eel, fish whose bloods differ more widely, but these fish were not available. At the same CO₂ pressures there was no significant difference between the behaviour of the two bloods. At high CO₂ pressures (50 mm. Hg) the bom-bom had a slight excess over the haimara, but the CO₂ content was definitely of the same order in both (20 vol. per cent. as opposed to 18 vol. per cent.), and the change in pH was also found to be of the same order although, if the results are to be trusted, there is some suggestion that the curves (see Fig. 13) do not follow quite the same course.

The oxygen capacity of the blood of the fish examined differs very widely, and also varies very much from one individual to another. This partly depends on the efficiency with which the blood is drawn, but there is manifestly a considerable natural variation over and above this. Variations were found from 4.4 vol. per cent. in one bom-bom (its usual capacity was in the neighbourhood of 10 vol. per cent.) to 22.25 vol. per cent. in the hassa, and there seems to be some indication that high oxygen capacity is to some extent correlated with reduced CO₂ effect on the oxygen content, suggesting that the haemoglobin plays some part in the CO₂ carriage.

Fig. 14 shows the relationship between the amount of CO₂ carried by the bloods of different animals at 25 mm. Hg CO₂ pressure and the corresponding pH of the blood. The data have been derived from various sources, those for the marine fish and the carp being obtained from figures given by Root (1931) and Wastl (1928) and being based on theoretical considerations. The curve distinguished by the circles was constructed from the Henderson-Hasselbalch equation and would represent the CO₂ carried at 26° C. by human blood at different pH's under 25 mm. Hg pressure of this gas, assuming the value of pk₁ in the equation to be 6.1. It is evident from a
consideration of the curve for the blood of different animals that the more acid the blood, the less CO₂ it can carry at constant CO₂ pressure. This is only to be expected, since it is believed that the majority of CO₂ is carried in blood in the form of bicarbonate, as is assumed in the Henderson-Hasselbalch equation. However, in the acid range, the two curves diverge widely, which suggests that some other factor may be entering into the situation. It is clear that the points for the haimara and the bom-bom lie at some distance above the theoretical curve, and that although the bloods of these fish carry less CO₂ than those of other fish, yet they are carrying more than they should according to theory, if they are to be compared with the above-

![Graph](image)

Fig. 13. Relationship between the CO₂ pressure and the resulting pH of the blood. Curves for mackerel, sea robin and toadfish obtained from data given by Root (1931). The figures opposite the points indicate the CO₂ content of the blood in vol. per cent.

mentioned human blood and depend on simple bicarbonate buffering. The difference between the two curves is almost certainly significant and beyond the range of experimental error, and, although only two fish have been examined and those somewhat incompletely, it would seem to point to a definite difference between the observed and calculated results. Similar discrepancies have been recently observed by Margaria and Green (1933) in mammalian blood and attributed by them to a direct CO₂-Hb compound. It would be extremely interesting to investigate the blood of such fishes as the haimara and the bom-bom under more fully controlled conditions¹ and in more detail, since the results of the few experiments here

¹ E.g. in the experiments here described the pH measured was that of the dialysate of the blood, whereas the CO₂ content was determined on the whole blood.
reported suggest that these bloods show the same phenomena on an exaggerated scale. Indeed the \( pK_a \) values in the Henderson-Hasselbalch equation derived from the data for the haimara and bom-bom work out between 5.0 and 5.6, which are from 0.5 to 1 unit lower than any previously recorded, so that one is tempted to question the validity of the results. If the data are eventually shown to be correct, then it would seem likely that these fish with acid blood are enabled to carry their CO\(_2\) away by the direct intervention of haemoglobin, thus using a different method of buffering from that normally used by mammals, and one which is apparently less efficient.

In one experiment on the bom-bom (Fig. 15) the CO\(_2\) contents of oxygenated and reduced blood were compared, and it at once became evident that there was no significant difference. This result agrees with expectation in that the blood being acid contains haemoglobin which is presumably behaving as a base, and therefore the relative strengths of haemoglobin, and oxyhaemoglobin acids do not enter into the question, as they do in blood which is on the alkaline side of the iso-electric point of haemoglobin.

It is unfortunate that the blood of the hassa and electric eel were not available for investigation of their CO\(_2\) content, since in them the oxygen capacity was con-
considerably higher than in the fish examined. This fact, if the O₂ capacity depends on
the haemoglobin content, might be expected to give their bloods greater buffering
power, and enable them to cope with the high CO₂ tensions to which they must be
subjected. Only a further investigation of the buffering powers of these acid bloods
can elucidate the situation.

![Graph](image)

**Fig. 15. CO₂ absorption curve for bom-bom blood.**

- Oxygenated blood.
- Reduced blood.

**DISCUSSION.**

The range of conditions which affect the respiration of fish living in the rivers,
creeks and swamps of British Guiana is exceptional, and, as might be expected, a
parallel range of respiratory behaviour is found in the fish. In the rivers, where there
is little CO₂ and a high oxygen content, their respiration is entirely carried out by
means of gills, and the oxygen dissociation curves for their blood more or less con-
form to the types found by Root (1931) and by Krogh and Leitch (1919) in marine
fish. That is to say that there is nothing remarkable about the dissociation curves at
negligible CO₂ pressures, but that the blood is very adversely affected as an oxygen
carrier by any CO₂ present, and consequently the river fish are unable to penetrate
into the more stagnant waters of some of the creeks and the swamps, where there is
often a very large quantity of dissolved CO₂. Such waters were found to be in-
habitad by fish whose blood was very much less affected by CO₂ (see Fig. 16). Even
these fish must frequently meet conditions where the blood could not be saturated
when in equilibrium with the water, and in them aerial respiration, either facultative
or obligatory, is almost the rule. Certain small fish, the huri (*Hoplias malabaricus*
(Bloch.), young) for example, overcome the difficulty by living with the mouth in
the surface layer of water which is presumably well oxygenated and nearly free from CO₂. It is interesting to notice that the huri is really the young form of the haimara, but their habits and appearance are so different that the Indians know them by different names. The young are found in swamps and pools in the forest, and the adult fish in the faster rivers and creeks. In the absence of CO₂ the dissociation curves for the blood of swamp fish were essentially similar to those of the river fish. There was no evidence that the blood was specially adapted to saturation at low O₂ tensions, as Krogh and Leitch found to be the case for the carp, pike and eel. There are, however, two points worth consideration in this connection. In the first place water with low oxygen and low CO₂ seldom occurs, and secondly the fish in Guiana live and were studied at a temperature of about 28° C., while those studied by Krogh and Leitch were at about 15° C. Guiana fish blood might easily behave like carp blood if subjected to the same temperatures.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Oxygen capacity</th>
<th>Percentage saturation at 40 O₂ mlf CO₂</th>
<th>Percentage saturation at 40 O₂ 25 CO₂</th>
<th>Loss in percentage saturation due to 25 mm CO₂</th>
<th>Locality of fish etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paku</td>
<td>10 78</td>
<td>90</td>
<td>42</td>
<td>48</td>
<td>Active fish of open river, especially near the falls</td>
</tr>
<tr>
<td>Haimara</td>
<td>6 53</td>
<td>82</td>
<td>54</td>
<td>28</td>
<td>Active fish of rapidly flowing creeks and rivers</td>
</tr>
<tr>
<td>Yakatu</td>
<td>11 3</td>
<td>83</td>
<td>59</td>
<td>24</td>
<td>Active river fish</td>
</tr>
<tr>
<td>Baiara</td>
<td>10 22</td>
<td>85</td>
<td>63</td>
<td>22</td>
<td>Active river fish</td>
</tr>
<tr>
<td>Bom-bom</td>
<td>10 60</td>
<td>87</td>
<td>74</td>
<td>13</td>
<td>Sluggish river fish—tends to live in mud</td>
</tr>
<tr>
<td>Hassa</td>
<td>18 14</td>
<td>91</td>
<td>79</td>
<td>12</td>
<td>Air-breathing fish, chiefly found in trenches and stagnant water, Sluggish habits</td>
</tr>
<tr>
<td>Electric eel</td>
<td>19 75</td>
<td>87</td>
<td>80</td>
<td>7</td>
<td>Sluggish fish found in muddy creeks, Can breathe air</td>
</tr>
</tbody>
</table>

Fig. 16.

Besides the oxygen and the CO₂ content of the water its hydrogen-ion concentration may be very important as a limiting factor in the distribution of fish, since there is a very considerable range between river water (pH 6.7) and the swamp water (pH 4.0). The yarrow, for example, which is a typical swamp fish, was adversely affected by river water. Experiments in transferring river fish to swamp water were not tried, but apart from the high CO₂ and low oxygen contents, the acidity might be expected to have harmful results on the fish. The yarrow, however, shows a certain adaptability to changes in pH. Its respiratory control mechanism is very little affected by the hydrogen-ion concentration of the water between pH 4 and 5, and it is not till the pH rises above 6 that any deleterious effects are noticed.

As far as the measurements made on the pH of the bloods of the fishes examined will allow any conclusions to be drawn, it would appear that the pH in river fish is approximately the same as that of the surrounding water, but in the swamp fish it
is probably considerably higher than that of the water. The lowest pH for oxygenated blood in the absence of CO₂ was found in the electric eel, where a pH of 6.2 was registered. It is possible that in more acid waters lower values might be obtained, because there appears to be considerable variation from one individual to another and the delicacy of pH control found in the mammals has manifestly not been reached by the fish. The pH of the blood cannot be put forward as the sole cause of the small effects of CO₂ on its oxygen-combining power, although it may play its part.

The fact that the blood of these fish is acid, and probably on the acid side of the iso-electric point of their haemoglobin, leads to many interesting questions. The buffering power of the bloods is considerably reduced as compared with that in frogs and mammals, but in those fish examined the loss of CO₂ carrying power is apparently not so great as would be expected on theoretical grounds, if the mechanism of buffering which holds for mammals is true for these fish. The data, if they can be confirmed, would suggest that some other mechanism is brought into action, and that mechanism is possibly the direct combination of haemoglobin with CO₂, in which case a possible explanation is afforded for the high oxygen capacity of the blood of the hassa and of the electric eel, since the efficiency of this second type of buffering mechanism would depend greatly on the amount of haemoglobin present. This of course assumes that their high capacity is correlated directly with the haemoglobin concentration. Experiments with a haemoglobinometer indicated that this was at any rate approximately true in the case of the electric eel. High haemoglobin content would therefore be doubly advantageous to the fish in swamp waters. Other things being equal, it would mean a greater supply of oxygen in the body, and a greater buffering power of the blood to deal with excess CO₂, so that visits to the surface would need to be less frequent. In this connection it is interesting to notice that the hassa (oxygen capacity up to 22 vol. per cent.) only comes to the surface, on the average, once in ten minutes. The time spent below the surface, of course, depends on a variety of factors. It depends, for example, on the amount of air taken down, the efficiency with which that air is used, the volume and oxygen capacity of the blood, and on the metabolic rate of the fish. It is also interesting to notice that there is evidence of high oxygen capacity of the blood being correlated with lessened CO₂ effect, although this was not always observed. Considering the adverse effects of CO₂ and acidity on the oxygen-carrying powers of the bloods of other animals it seems remarkable that the oxygen capacity of the hassa and the electric eel, for example, should reach such high values.

Root, in his work on marine fish, noticed that the blood of the mackerel, an active free-swimming fish, had a high oxygen capacity, and from his data for other fish was able to correlate sluggish habits with blood of low oxygen-carrying power. In the case of the fresh-water fish examined this correlation ceases to hold good, and the oxygen capacity seems to be correlated more with the nature of the environmental conditions than with the swimming habits of the fish.

Certain light is thrown on one or two problems of general biological interest by the results described in this paper. In the first place a possible explanation is
afforded for the almost exclusive presence of oxygen in the swim-bladders of certain
deep-sea fish. If the blood of the fish conforms to the types investigated by Root
(1931) or to that shown by the paku, and it comes into contact with CO₂, then
oxygen will be evolved. Apart from the red gland, the swim-bladders of fish are
relatively poorly supplied with blood. It is true that the metabolism of their walls is
probably also low, but even so, it seems quite likely that CO₂ should collect in the
cavity of the bladder in sufficient amount to liberate even a small quantity of oxygen
from the blood circulating through the rete mirabile, and in this way it seems
possible to understand how, slowly but surely, the bladder would become filled with
almost pure oxygen. The nitrogen would not increase in the bladder since it would
be in equilibrium with its solution in the blood, which would in turn be in equili-
brium with the nitrogen in the outside water. The oxygen pressure on the other
hand would rise till it balanced the hydrostatic pressure on the swim-bladder, and
the nitrogen would become a proportionately smaller constituent of the gas in the
bladder. Although the oxygen capacity of these fish is lowered by CO₂, it has yet to
be proved that increased oxygen pressure, possibly to several atmospheres, will not
cause recombination of the O₂ with the blood, but the data available suggest that
this recombination is unlikely. It must, however, be remembered that the pressure
of gas in the swim-bladder is often enormous in comparison with the pressures for
which the data have been obtained. The apparently secreting cells, described in
certain red glands, may conceivably be concerned with CO₂ production and regula-
tion.

Another point upon which the present work may throw light is the question of
whether fish can oxygenate water in which they live. The results here described
show clearly that for some fish this is certainly possible. The yarrow does not
oxygenate the water in which it is placed, because as soon as the conditions prevail
when it would be possible for it to do so, it shuts down its gills and prevents oxygen
escaping from its blood in that way (see Fig. 11). But on the other hand take, for
example, Lepidosiren paradoxa, an air-breathing fish in which the male of the species
has during the nesting season certain highly vascular pelvic filaments, which it is
incapable of removing from contact with the water. Put this fish under the same
conditions as the yarrow, and supposing that its blood be similar to that of the
yarrow, it cannot help losing oxygen to the water, and might very well go on losing it
till the surrounding water contained from 1 to 2 c.c. of oxygen per litre. The
filaments could in this way oxygenate the water in the nest of this mud-fish so that
the eggs and young would have enough oxygen for their development. There is
another possibility which would make the task of the fish even easier. So far it has
been assumed that the blood of the Lepidosiren is unaffected by CO₂. It might not
be. It might conform to the marine fish or paku type, in which case the CO₂ in the
water of the nest would enormously aid in the liberation of oxygen. However, on
general grounds it is rather unlikely to be so greatly affected by CO₂ as this, but even
if affected to the extent to which mammalian blood is affected, then considerable
quantities of O₂ would be liberated by this mechanism.
SUMMARY.

1. The blood of the fish of the rivers and swamps of British Guiana is not all equally affected by CO₂. A regular series can be shown to exist between the paku (Myleus setiger Mull and Troschel), an active river fish whose blood is considerably affected, and the electric eel (Electrophorus electricus (L.)), which is an air-breather, frequenting relatively stagnant and poorly oxygenated water, and whose blood is almost independent of the presence of CO₂.

2. The blood of all the fish examined was found to be acid, generally about pH 6-7.

3. The CO₂ combining power of the blood of the fish examined was found to be very much lower than in other animals, but higher than might be expected on theoretical grounds. The possibility of another type of buffering is suggested.

4. In the one fish examined (the bom-bom, Pterodoras granulosus (Val.)) the CO₂ contained in reduced blood was found to be no higher than in oxygenated blood.

5. In spite of the low oxygen content of some of the waters, no fish examined showed a haemoglobin with any markedly increased affinity for oxygen, such as might enable it to be saturated more easily at low pressures.

6. The yarrow (Erythrinus erythrinus (Schn.) or Hoplerythrinus unitaeniatus (Spix.)) was found to be a fish which could either use its gills or take in bubbles of air into its air-bladder as a means of respiration. This fish has been studied in detail and the conditions controlling its respiration are described. Three factors are concerned, pH, oxygen content of the water, and CO₂ content of the water. Of the three the last is most often the limiting factor.

7. Carbonic anhydrase was found in the blood of all the fish examined for its presence, which include practically all those mentioned in this paper.

8. The bearing of these results on certain problems of general biological interest is discussed.

REFERENCES.