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THE FUNCTION OF HAEMOGLOBIN IN RELATION TO THE MAINTENANCE OF NEUTRAL BUOYANCY IN *ANISOPS PELLUCENS* (NOTONECTIDAE, HEMIPTERA)

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INTRODUCTION

Among insects, haemoglobin is known to occur only in certain chironomid larvae, in *Gastrophilus* larvae, and in the water bugs *Macrocorixa geoffroyi* and those belonging to the subfamily Anisopinae. Only in the chironomid (Walshe, 1950) and *Gastrophilus* larvae (Keilin & Wang, 1946) is much known of its function and properties. A useful summary of the characteristics of haemoglobin in these insects has been provided by Buck (1964). In *Gastrophilus* (Keilin, 1944) and in *Anisops* and *Buenoa* (Anisopinae) (Hungerford, 1922; Poisson, 1926; Bare, 1929) haemoglobin is found in certain richly tracheated cells in the abdomen; in the first it may also occur in other tissues in smaller amounts. *Anisops* and *Buenoa* are the only insects known to possess haemoglobin in both larval and adult stages, but its function and properties in these genera have remained unexplored. Both are remarkable for their habit of remaining poised in mid-water in more or less neutral density for a large part of each dive (Bare, 1926, 1929; Jaczewski, 1936; Kaiser, 1940), and the suggestion that this ability is due to the possession of haemoglobin has been made by Bare (1929), Hutchinson (1953) and by Hungerford (1958). Miller (1964*a*, *b*) described some preliminary experiments whose results were in agreement with this hypothesis, and further supporting evidence is presented here from recent work carried out at Makerere College on *Anisops pellucens* in Uganda. The haemoglobin in this species is shown to have a strikingly low affinity for oxygen and this property permits unloading of the pigment during a normal dive of about 5 min. duration even in well-aerated water. The rate of depletion of the external gas store is thereby diminished and the bug is thus enabled to remain in or near neutral buoyancy for several minutes. The haemoglobin may therefore be looked upon as an unusual type of store which is regularly called upon during each dive, and not one which is used only under conditions of severe oxygen deprivation. Its use enables the bug to exploit the mid-water zone, a habitat not readily available to aquatic insects other than the larvae of the nematocerans, *Chaoborus* and *Monochlonyx* (Damant, 1924).

MATERIAL AND METHODS

Adult *Anisops pellucens* Gerstaecker were used in all the experiments reported here. (Length 11–12 mm; weight 50–70 mg.) The bugs were collected in large numbers from Kajansi experimental fish farm near Kampala, Uganda. They were

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kept in the laboratory in aquaria and fed on fish fry, small tadpoles and various small aquatic insects.

Spectroscopic examination of the pigment in the intact bug was first attempted using a Zeiss ocular spectroscope. However, the opacity of the abdomen made it virtually impossible to transmit adequate light for examination. After removal of the abdominal tergites it was possible to examine the absorption bands, but then only with difficulty.

In some experiments the pigment was extracted by grinding up the abdomen in distilled water and then filtering and centrifuging to get a clear pink solution. Gas mixtures were bubbled through the solution and the appropriate absorption bands for reduced and oxygenated haemoglobin were recognized. However, continued agitation led to precipitation of the haemoglobin in long fibres after which further tests could not be carried out.

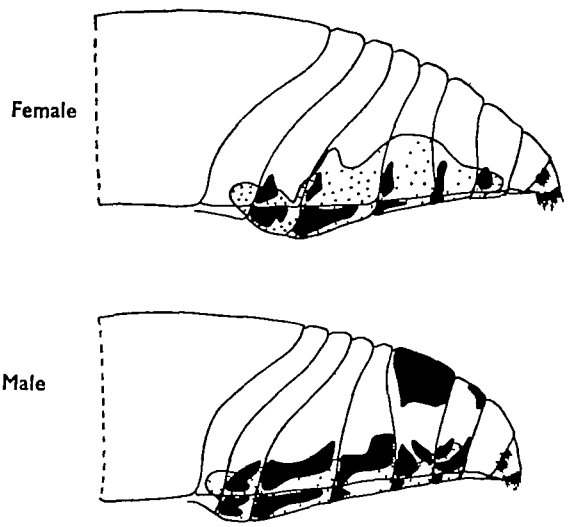

Fig. 1. Lateral view of the abdomen of a female and a male *Anisops pellucens* to show the areas of black pigment and those parts (stippled) where haemoglobin can be seen through the transparent cuticle.

Since the properties of haemoglobin often change after dilution and removal from cells (cf. Manwell, 1964) and the formation of methaemoglobin may be hard to avoid (Keilin & Wang, 1946) an alternative method for determining the state of the pigment was adopted: it depended on observations by eye of the change in colour of reflected light with the uptake and release of oxygen. The pigment appeared pink-red when fully oxygenated and purple-black when reduced. Intermediate saturations were estimated by comparisons with these two end-points and with artificial solutions of various shades. Since the colour change takes place very rapidly, the comparisons could be made a few seconds after each other by flushing nitrogen and then air through the gassing chamber. Reasonable estimates of 90, 75, 50, 25 and 10% saturations could be made in this way, and with less certainty some intermediate points were determined. The assumption was made that the half-way colour change corresponded to half saturation.

This method is clearly less accurate than conventional spectrophotometric methods, but has the great advantage that it can readily be carried out on the intact bug, even when the bug is freely swimming in a small aquarium where it can be followed with a low-power microscope. It gives reasonably exact information about the position of the dissociation curve, but the shape of the curve cannot be accurately determined. For example, Fig. 4 suggests the curve is sigmoid (that haem-haem interactions are taking place), but the sigmoid character in fact probably only illustrates the difficulty of making estimates near 0 and 100% saturation.

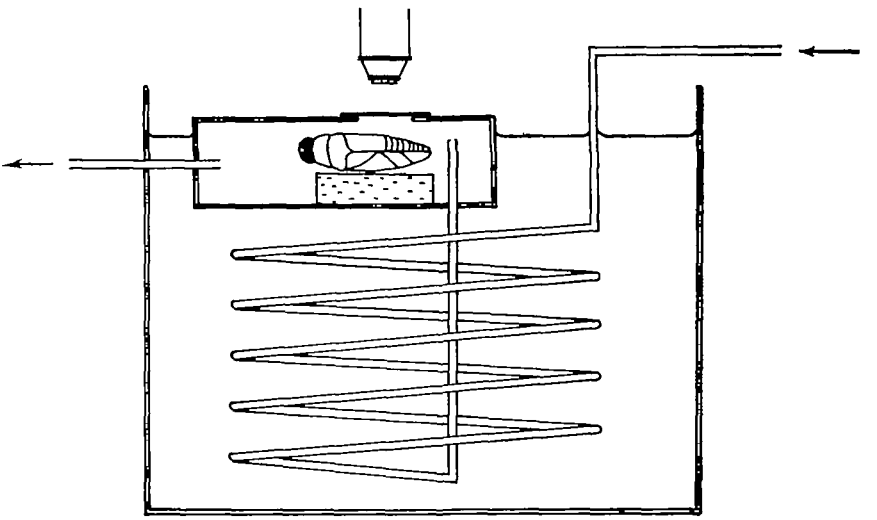

Fig. 2. Diagram of the chamber used for passing gas mixtures over *Anisops pellucens*. The gases are first piped through the water bath surrounding the chamber.

The haemoglobin cells are less obscured by black pigment in females than in males (Fig. 1); the former were therefore used in most experiments. In order to determine the dissociation curve, the wings were removed from a female which was then attached to a plasticene base in a small gassing chamber (capacity about 10 ml.) in such a way that the side of the abdomen could be examined under the microscope. Gas mixtures were either prepared beforehand and stored over acid-saline or they were mixed at the time of the experiment by passing gases through calibrated manometric flow-meters. Nitrogen was bubbled through a pyrogallol trap to remove traces of oxygen. Samples of gases were removed at intervals from the gassing chamber and analysed at constant pressure as a check on the mixtures. The gassing chamber was contained in a large water bath through which the test gases were piped before they arrived in the chamber. In this way the gases, which were delivered at 200–500 ml/min., reached the temperature of the water, even when this was well above ambient (Fig. 2).

Dive durations in populations of bugs were measured by counting the number of surfacings per 15 min. Normally between twelve and twenty-five bugs were used at a time, but tests were also made on single bugs.

Experiments were carried out at 24–25°C unless otherwise stated. In calculating gas pressures from the proportions determined by analysis, account is taken of the fact that the barometric pressure at Makerere College is 660 mm. Hg (cf. Jones, 1964). The Ringer used was that given by Pantin (1948).

MORPHOLOGY OF THE RESPIRATORY TREES

The external features of the respiratory system of *Buenoa* have been described by Bare (1929) and of *Anisops* by Poisson (1926). The ventral airstore is enclosed under long hairs as in other Notonectidae and it is in continuity with a bubble trapped between the leg bases which extends anteriorly between the head and prothorax and is continued under the pronotum and under the wings. All abdominal and thoracic spiracles therefore open into the same air space as in *Notonecta* (Ege, 1915). The outer dorsal surface of the forewings of *Notonecta* is covered in small bristles, about 100μ long, which are flattened and broader at the tip than at the base. They entrap a film of air which, as Ege noted, is in contact with the rest of the air spaces. In *Anisops*, however, the transparent wings bear no bristles and carry no layer of air externally, but air is trapped under the wings giving them a silvery appearance. The air–water interface in *Anisops* is therefore limited to the ventral stores. The area of gas store exposed to the water is 0·122 mm^2/mg. in *A. pellucens*. In *Notonecta* the same stores have a similar exposed area relative to weight of 0·132 mm^2/mg. (Miller, 1964*b*), but when the gas layer on the wings is included the figure is 0·5 mm^2/mg. (de Ruiter, 1952). Exchange between the gas stores and the water is therefore likely to be more effective in *Notonecta* than in *Anisops*, particularly since the ventral abdominal store in both genera is shielded by a row of long bristles which interposes a continuous hydrofuge cuticular barrier between the gas and the water and may thus reduce the rate of diffusion between them. The airfilm on the wings of *Notonecta* is one factor making this genus more positively buoyant than *Anisops*.

The volume of air trapped beneath the wings of *Anisops* is small and probably has little respiratory significance. The presence of an air-filled cavity is essential, nevertheless, to enable the bug to spread its wings in flight immediately it leaves the water. This it can do by swimming through the meniscus, as do the Corixidae. Surface tension may thus help wing unfolding in a way directly comparable to the spreading of the long bristles, which cover the ventral abdominal store, when the insect reaches the water surface. Such a mechanism makes it possible for *Anisops* to leave and enter open waters without the need to crawl up or down plants and is probably an important feature enabling it to colonize fresh bodies of water rapidly (cf. Kaiser, 1940).

The abdominal spiracles (spiracles 5–9) are covered by large white sieve plates and their atria lead directly into several broad tracheae. Each of these supplies a 'tree' of several hundred haemoglobin-filled cells. The organization of the 'tree' has briefly been described by Bare (1929). A large trachea (the trunk) proceeds through the centre of the tree giving off radial branches on all sides which proceed to the periphery and in turn give off many side branches. The organization is comparable to the 'centro-radial' organization of flight muscle tracheation (Weis-Fogh, 1964), and indeed the richness of secondary branching and the density of tracheoles in the haemoglobin

cells strongly parallels that found in flight muscle. Each haemoglobin cell (approx. $20 \times 80\ \mu$) is apparently penetrated (or indented) by a small trachea which travels through the cell giving off numerous tracheoles as it proceeds towards the peripheral pointed end (Fig. 3). Near the point of 'entry' of the trachea there is a marked diminution in bore and the taenidium abruptly becomes almost invisible under the light microscope. When gentle pressure is applied to a tree on a slide, bathed in Ringer, haemoglobin is expressed from the cells in spherical membrane-bounded vesicles. The cells with their tracheal skeletons then appear collapsed and shrunk; their nuclei and numerous granules can readily be seen. In distilled water both the cells and the expressed vesicles swell up and burst, releasing the haemoglobin into solution.

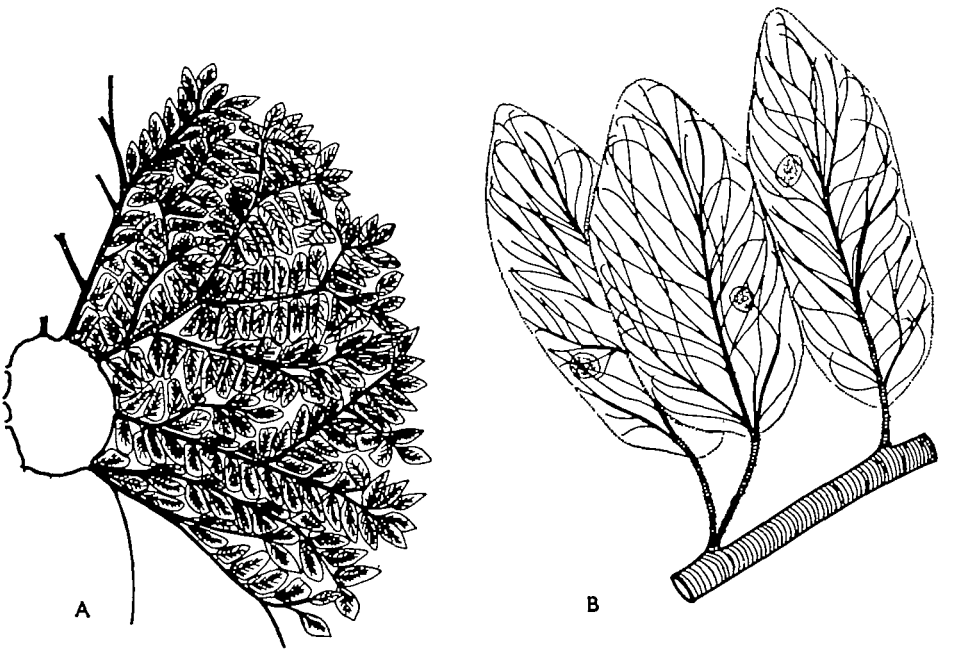

Fig 3 A. Part of a 'tree' of haemoglobin-filled cells in *Anisops pellucens*. The central trunk is shown with a few radiating secondary branches. B. Three haemoglobin-filled cells showing a small part of the tracheolar mesh by which they are invaded. Each cell is between 60 and 100μ long.

A small trachea leaves dorsally from each tree to join the slender (10–25 μ diameter) lateral trunk which proceeds into the thorax. A larger trachea (40–70 μ diameter) leaves from the same point and supplies either the gonads or the gut, in different segments. Thus oxygen leaving the pigment and entering the tracheal system may supply the abdominal organs, or it may proceed to the thorax through the slender lateral trunk, or again it may return to the external abdominal gas store and then enter the thorax via the thoracic spiracles. Experiments will be described below which indicate that the last of these routes is the most important—a route which offers the least resistance to diffusion.

The general arrangement of tracheae and haemoglobin cells is similar to that found in *Gastrophilus* although in that species the haemoglobin cells are up to 400 μ in length (Keilin & Wang, 1946).

RESULTS

Oxygen dissociation curve. A curve was plotted using the colour change of the pigment as an index of the percentage oxygen saturation as already described. The results, shown in Figure 4, are derived from four bugs which were treated with various gas mixtures given in a random order. The curve shows the state of the pigment in equilibrium with different gases in the external store, and not with those in the immediate vicinity of the haemoglobin cells. However, since the haemoglobin trees lie close to the spiracles and are supplied by an abundance of tracheae, the difference in gas tensions is probably small. From the curve a P_{50} value for the haemoglobin of about 28 mm. Hg partial pressure at 24° C. and in the absence of added carbon dioxide can be derived.

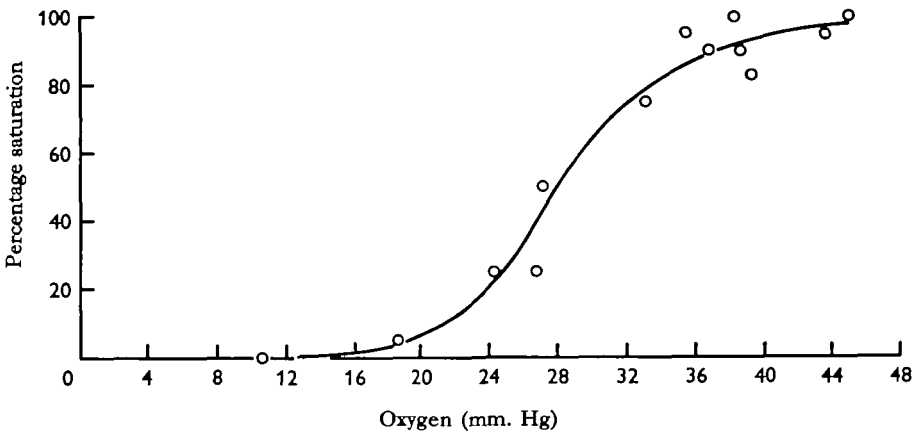

Fig. 4. The relationship between the ambient P_{O_2} and the percentage saturation of the haemoglobin of intact *Anisops pellucens*.

State of the haemoglobin during a dive. Six bugs at a time were narcotized with carbon dioxide and then attached to small plasticene pedestals in a beaker in such a way that their pigment could be observed under a microscope. They were allowed to recover for 15–30 min. and then covered with water. The time taken for the first sign of darkening of the pigment was measured, and also the time for complete darkening (the purple-black of desaturated pigment). As soon as the water level was lowered so that the bugs were again in air, the pigment flushed pink-red. The colour changes were the same as those observed in the bug treated with various gas mixtures. The time taken to darken was variable in different individuals, and depended on the amount of struggling and the number of swimming strokes that the bug attempted. In active bugs, darkening commenced in less than 1 min. and was complete in 2 min. In quiescent bugs, it did not start for 3 min. and was complete after about 5–6 min. Stirring the water or bubbling air through it did not affect the time taken to darken. These times correspond well with the over-all duration of a dive in an unrestrained bug and suggest that the haemoglobin is unloaded during a normal dive. This conclusion was confirmed by following the free dive of a single bug in a small aquarium with a binocular microscope and observing that the darkening of haemoglobin com-

menced 1–2 min. after the bug left the surface, at about the start of the phase of near-neutral buoyancy.

The low affinity for oxygen of the haemoglobin of *Anisops* therefore seems well adapted to allow unloading of the pigment during every dive even in well-aerated water. That this contributes to the maintained neutral buoyancy of the bug in mid-water is shown by some of the experiments to be described below.

The effect of pH change and carbon dioxide on the dissociation curve. In order to test for a possible Bohr effect, the abdominal tergites were removed from several bugs and their abdomens were floated on solutions of Ringer buffered at different pH values. In this way the Ringer bathed the outside of the haemoglobin cells. Ringers at pH 7·0, 6·0 and 4·0 had no effect on the dissociation curves (Fig. 5).

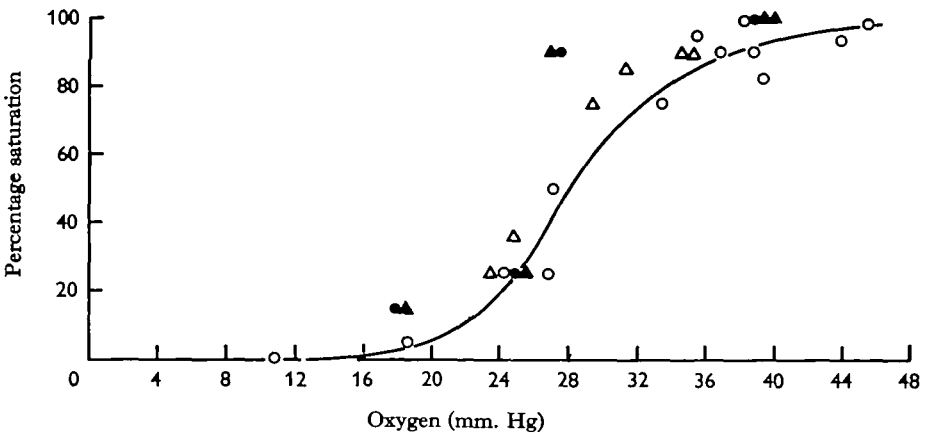

Fig. 5. The relationship between the ambient P_{O_2} and the percentage saturation of the haemoglobin of *Anisops pellucens*. ○ – ○, intact bugs; △ – △, intact bugs in 20 mm. Hg CO_2; ● – ●, abdomen with haemoglobin cells exposed to Ringer buffered at pH 7·0; ▲ – ▲, abdomen with haemoglobin cells exposed to Ringer buffered at pH 4·0 or pH 6·0.

Since the haemoglobin is intracellular it may not have been affected by the Ringer which was applied externally. In a second series of experiments, therefore, known amounts of carbon dioxide were added to mixtures of nitrogen and oxygen and intact bugs were treated with these mixtures in the gassing chamber as described above. The presence of carbon dioxide (up to 137 mm. Hg) had no effect on the position of the dissociation curve (Fig. 5). It seems probable that since oxygen reaches the pigment so readily through the tracheal system, there would be no barrier to the penetration of carbon dioxide. In common with the haemoglobin of several other invertebrates (e.g. *Gastrophilus*, some species of *Arenicola* and *Eupolymnia* (see Table 1 in Manwell, 1964)), that of *Anisops*, therefore, appears to have no Bohr effect.

The effect of temperature on the oxygen dissociation curve. In order to measure the temperature of the bug accurately a small thermocouple was introduced into the thorax of the bug in the gassing chamber. A thermometer was stationed nearby so that the temperature of the gases as they arrived in the chamber was known. The water in the bath around the bug was then heated or cooled and the thermometer showed that the gas entering the chamber was at the same temperature as the water.

With increase in temperature there is a marked shift of the curve to the right (Fig. 6). For a 10° C change the P_{50} value alters by about 13–15 mm. Hg (25–35° C.) (Fig. 7).

The very marked effect of temperature on the position of the dissociation curve is probably accounted for in part by the normal influence of temperature on the properties of haemoglobin and in part by an alteration of the metabolic rate. At increased rates of oxygen consumption, the difference between the ambient oxygen tension and that in the immediate neighbourhood of the haemoglobin cells is probably increased thereby contributing to the shift of the curves to the right.

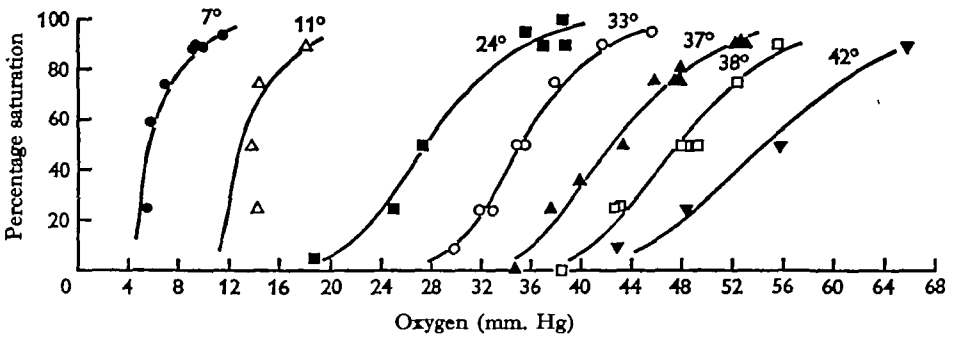

Fig. 6. Relationships between the ambient P_{O_2} and the percentage saturation of the haemoglobin of *Anisops pellucens* at various temperatures.

The effect of carbon monoxide on the dive performance. Preliminary experiments on the effect of carbon monoxide have already been reported (Miller, 1964*b*). They were repeated and extended here and checks were carried out to ensure that carboxyhaemoglobin was in fact formed under the experimental conditions.

Carbon monoxide was formed from the action of sulphuric acid on sodium formate. It was mixed with air or nitrogen in known amounts and stored over acid-saline. In different experiments, mixtures of 6, 12 and 25 % carbon monoxide in air were passed over the water in a closed aquarium containing bugs. When they surfaced the bugs filled their ventral airstore from the carbon monoxide mixture. As reported earlier, this resulted in a shortened dive in which the phases were telescoped, or frequently in one which was terminated by re-ascent while the bug was still in positive buoyancy (phase 1). Altogether thirty-two tests were conducted with about twelve bugs each; in air the average dive duration per bug was 4·7 min., while in all carbon monoxide mixtures it was 0·85 min. Some representative values are shown in Fig. 8. There was no significant difference in the dive duration in 6, 12 and 25 % carbon monoxide.

Since gas mixtures of 25 % carbon monoxide in air reduce the oxygen content to about 16 %, separate experiments were carried out in which 16 % oxygen in nitrogen was passed over the aquaria; in these the dive duration was unaffected. Only when the oxygen content was reduced to 7–8 % was the dive appreciably shortened, in contrast to the experiments of de Ruiter, Wolvekamp, van Tooren & Vlasblom (1952) on *Notonecta*. This would suggest that much of the oxygen consumed during a dive is derived from haemoglobin in *Anisops*. In carbon monoxide the bugs were more active than in air because the whole of each dive was often spent in com-

pensating for positive buoyancy and the phase of neutral buoyancy was never reached. The consequent increase in oxygen consumption would in itself lead to a curtailment of the dive. However, maximally active bugs under normal conditions still perform dives of at least 2 min. duration. These considerations mean that while little value can be attached to the precise duration of a dive under carbon monoxide they cannot alone explain its drastic reduction; formation of carboxyhaemoglobin is probably the most important cause.

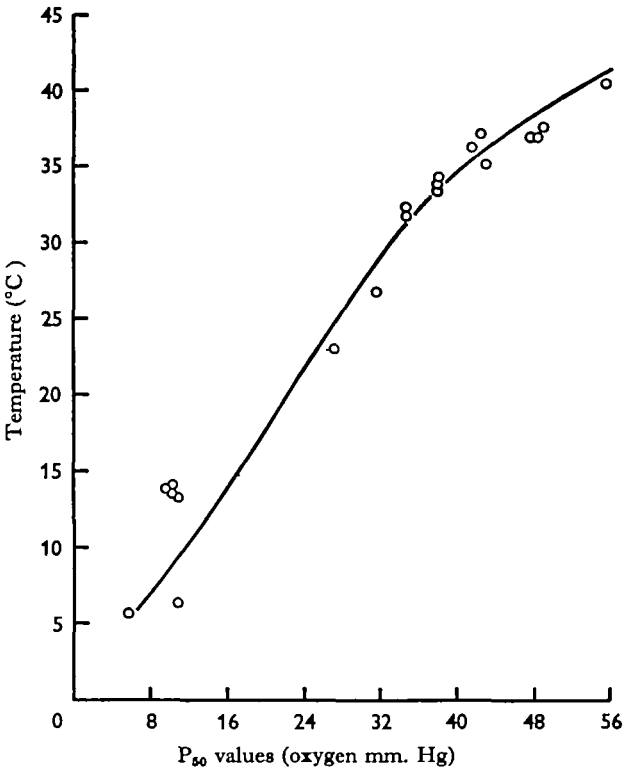

Fig. 7. Relationship between the temperature and partial pressure of oxygen at which the haemoglobin of intact *Anisops pellucens* is 50% saturated (P_{50}).

That carboxyhaemoglobin has been formed under these conditions is shown by a number of observations. First, when bugs were prevented from surfacing for several minutes under carbon monoxide their haemoglobin was found to be still pink-red in colour; the typical darkening did not occur. Secondly, the formation of carboxyhaemoglobin was checked on extracted solutions of haemoglobin with a spectroscope. The addition of sodium dithionite normally caused such solutions to darken and show the single broad band of reduced haemoglobin, but after bubbling through 12 or 6% carbon monoxide in air, dithionite had no effect on the colour and two strong bands were still visible with the spectroscope. Lastly, the colour change of the pigment in the intact bug in different mixtures in the gassing chamber was observed. Passing nitrogen through the chamber brought about a rapid darkening as before; however, when 6% carbon monoxide in nitrogen was passed through,

the pigment remained pink-red as in air. On returning to nitrogen the pigment darkened but less rapidly than normally.

These experiments show that, in the presence of 6% carbon monoxide in air, carboxyhaemoglobin is formed. They give no measure of the affinity of *Anisops* haemoglobin for carbon monoxide beyond showing that it is greater than that for oxygen.

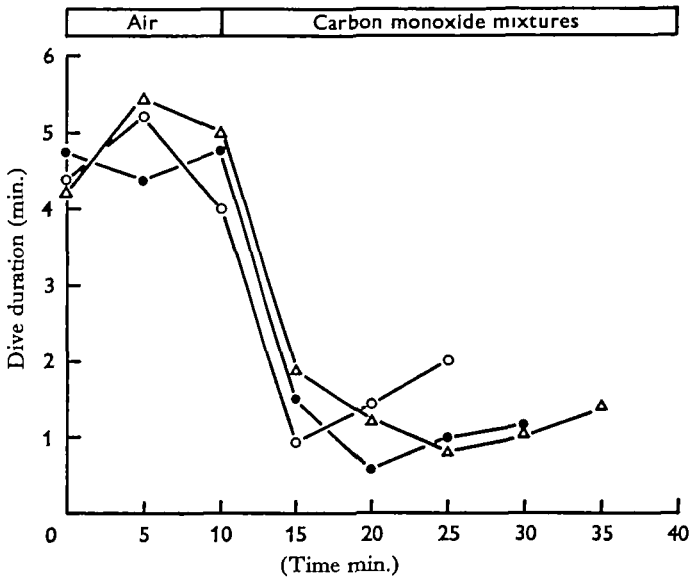

Fig. 8. The duration of dives of *Anisops pellucens* in air and in mixtures of carbon monoxide and air. Each point on the graph represents the average value from twelve bugs. △—△, 25 % carbon monoxide in air; ●—●, 12·5% carbon monoxide in air; ○—○, 6% carbon monoxide in air.

Miller (1964*b*) described the occurrence of a long period (up to 2 hr.) after treatment with carbon monoxide during which the dives gradually resumed their normal duration. The long after-effect is probably caused by carbon monoxide which has dissolved in the water and in the bugs' tissues and continues to affect the pigment. This period was reduced when bugs were transferred to fresh water immediately after treatment with carbon monoxide.

The fate of oxygen released from haemoglobin during a dive. Oxygen released from the haemoglobin may enter the haemolymph in solution or enter the tracheal system as a gas. In the latter case, it may then either supply abdominal organs, or proceed to the thorax in the narrow lateral trunk, or pass into the ventral airstore and then re-enter the tracheal system via the thoracic spiracles. In the absence of ventilating movements (which are rarely seen) the last route will probably be the most important since the wide diffusion pathway offers much less resistance than other routes, even though the partial pressure difference may not be so great as in tracheae leading directly to the tissues.

That this interpretation is correct is supported by a number of observations. When one spiracle is blocked with petroleum jelly and the bug is submerged, the haemoglobin normally supplied through that spiracle remains pink-red while the remainder

darkens. Several minutes later it too darkens, but this may not occur until the bug has been held under water long enough for asphyxiation to have occurred (cessation of all movement). Thus the bug cannot readily use the oxygen from its haemoglobin except via a spiracle. Similarly if the ventral airstore is divided by a transverse petroleum jelly block, then after the bug is submerged the haemoglobin anterior to the block darkens within 1–2 min., while that in the posterior region remains pink-red, again until after the bug has been asphyxiated. This method might allow one to measure the contribution made by a single haemoglobin tree to the total dive duration of a freely swimming bug, but this has not been undertaken so far. Since a group of haemoglobin trees can act independently in this way when its spiracle is sealed, and the groups are functionally connected only through the ventral airstore, the lateral trunk (10–25 μ in diameter), which joins anteriorly near spiracle 4, seems to play an insignificant part in the movement of gases from abdomen to thorax. The ventral airbubble which offers a diffusion pathway about 350 times greater in sectional area is therefore the main conduit between the haemoglobin and the thorax.

The metathoracic muscles which control the movements of the hind (swimming) legs occupy a volume greater than that of the flight muscles. They are probably the principal consumers of oxygen during a dive, since they are responsible for the compensatory swimming strokes. They are supplied by tracheae which arise mainly from spiracle 4 (the second abdominal spiracle) which opens under the metathoracic coxa, just anterior to the ventral abdominal airstore. They are thus well placed to receive oxygen from the haemoglobin store.

During a dive certain leg movements are intermittently seen which force a bubble of gas posteriorly from between the coxae into the abdominal store; the leg movements then stop and the bubble is sucked anteriorly again. Such movements bring about mixing of the air held more anteriorly with that in the external store and may accelerate the passage of oxygen anteriorly from the abdominal to the thoracic spiracles. It is apparently a method of ventilating the external air passages.

DISCUSSION

The experiments described here indicate that the haemoglobin of *Anisops pellucens* has a low oxygen affinity and unloads its oxygen during the course of every dive. The oxygen capacity of the pigment is not known, nor has the rate of oxygen consumption by the bug been measured; the actual contributions made by the pigment and by the external stores cannot therefore be calculated. However, the carbon monoxide experiments, which inactivated the haemoglobin and reduced the dives to about a fifth of their normal duration, suggest that the major part of the oxygen consumed comes from the pigment. During the first phase of a dive when the bug is positively buoyant, the external bubble is likely to be the main oxygen contributor; the partial pressure is probably reduced to about 36 mm. Hg during this period at which value the pigment starts to unload. This stage probably corresponds to the start of the midphase of the dive when the bug is at or near neutral buoyancy. The oxygen consumption will now be less since only occasional corrective swimming strokes are required. During the mid-phase the oxygen tension falls more slowly in the bubble as the oxygen store in the haemoglobin cells is given up, until

at about 20 mm. Hg the pigment is fully unloaded and only a small amount of oxygen remains in the external store. As this in turn is called upon, the bug becomes less buoyant; more corrective strokes are required and the oxygen consumption increases. The vicious circle is soon terminated by ascent to the surface, and a new dive commences. This hypothesis is summarized in Fig. 9.

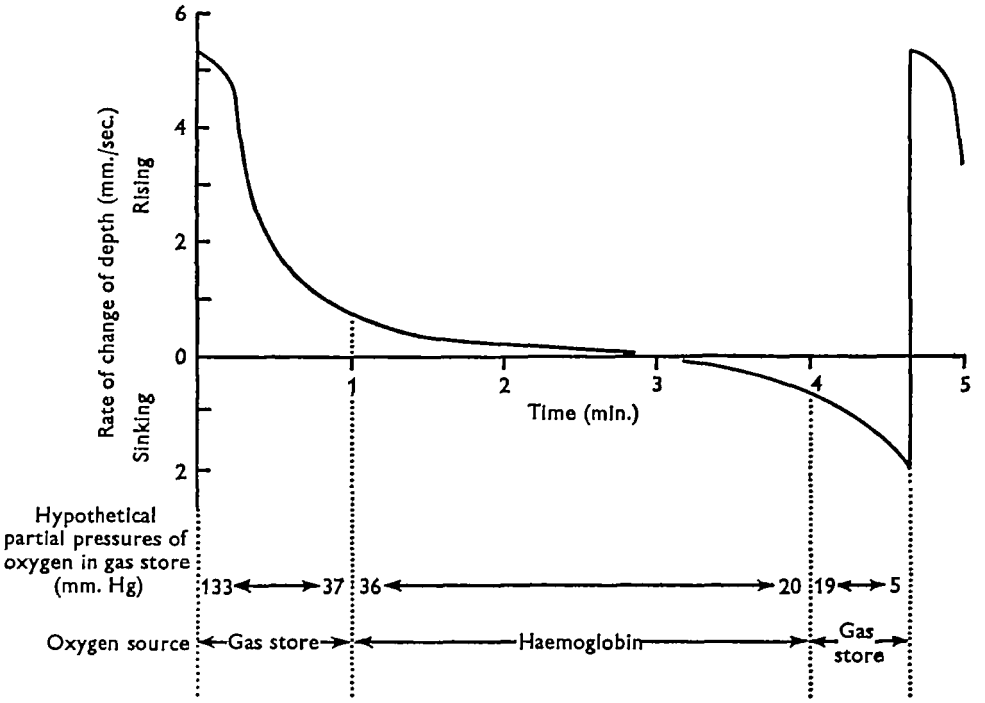

Fig. 9. Diagram representing the probable source of the oxygen consumed at different times during a dive of *Anisops pellucens* together with the hypothetical values of the P_{O_2} in the external gas store (partly based on Miller, 1964*b*).

When a small portion of a haemoglobin tree is placed in a drop of Ringer on an inverted cavity slide and examined in the gassing chamber as a hanging drop preparation, a vivid impression of the release and uptake of oxygen can be obtained from the expansion and contraction of the attached tracheae (provided their open ends are sealed with Ringer), as nitrogen and air are in turn passed through the chamber. The oxygen given up by the haemoglobin temporarily inflates the tracheae, while they are deflated when oxygen is again absorbed by the pigment. The movements take place within 1 or 2 sec. of admitting a new gas, but after a further few seconds the tracheae return to their original dimensions as a result of gaseous diffusion and tracheal resilience. The maximum volume change in a piece of trachea attached to thirteen haemoglobin cells was found in this way to be about 1000 μ^3, and from this it can be said that thirteen cells release *not less* than this amount of oxygen; in fact they probably release considerably more.

The haemoglobin of most invertebrates has a high affinity for oxygen. *Anisops* (and probably *Buenoa*, which has similar habits) therefore stand apart, and, assuming

that the value of 28 mm. Hg is near the true P_{50} of the pigment, a lower affinity seems to be known only in the terebellid *Eupolymnia* where the P_{50} is 36 mm. Hg at 10° C. and pH 7·2 (Manwell, 1959). Other respiratory pigments are known among invertebrates, however, with lower affinities for oxygen. While at 25° C. the P_{50} for *Anisops* haemoglobin is comparable to that for man at 37° C., full saturation of the bug's pigment seems to occur at a much lower P_{O_2} than in man. However, because of inadequacies in the method of estimating the percentage saturation of the pigment, errors are likely to be large in the neighbourhood of 0 and 100%.

A functional interpretation of the low affinity of *Anisops* haemoglobin can be made in terms of the dive behaviour and of the need to prevent rapid depletion of the gaseous stores. Some of the ecological consequences and benefits which accrue from exploitation of the mid-water zone, made possible by the possession of haemoglobin, have already been discussed (Miller, 1964*b*). It is clear, nevertheless, that the nematoceran larvae *Chaoborus* and *Monochlonyx*, which are also found in mid-water, are superior to *Anisops* since they are independent of the surface for renewal of their stores; they are permanently in neutral buoyancy; and they can exercise a positive control over their buoyancy and hence can adjust to different depths. Both when changing depths and when burdened with prey, which may be carried throughout several dives, *Anisops* is at a disadvantage compared with the dipteran larvae.

Keilin & Wang (1946) calculated that the haemoglobin of *Gastrophilus* could supply the insect with oxygen for 4 min. In *Anisops pellucens* the oxygen from the haemoglobin appears to be consumed or lost to the environment in less than 5 min., while in *A. debilis* all may be consumed in about 1–2 min. (Miller, 1964*b*). Although *Anisops* may lose oxygen to the surrounding waters when the ambient P_{O_2} is low, and may gain oxygen when the tension in the environment is high (physical gill action) the exchange is likely to be small compared with that in *Notonecta*. Physical gill action in *Notonecta* is believed to provide a considerable amount of the oxygen consumed when the bug is quiescent at temperatures below 17° C. (Ege, 1915). Ege showed that the invasion of oxygen takes place mainly into the gas layer held against the outside of the forewings: when this was removed the bug survived only 15 min. when prevented from surfacing, as compared with 7 hr. for a bug with airstores complete in well-aerated water. Popham (1962) argued that the amount of oxygen derived from the water in this way was insignificant in *Notonecta* above 17° C. although ventilating movements by the legs which sweep fresh water past the gas may considerably improve the conditions for exchange. However, these are not often seen under natural conditions (de Ruiter *et al.* 1952).

In *Anisops*, physical gill action probably contributes a negligible proportion of the oxygen consumed for four reasons: there is no superficial air-layer on the wings; the ventral abdominal airstore is shielded by a hydrofuge curtain of bristles; at 25° C., with active swimming strokes continuing to some extent throughout a dive, the oxygen consumption is probably high; and finally because the oxygen contribution from the haemoglobin may keep the tension in the stores higher than would otherwise be the case. For example, in the absence of haemoglobin in *Dytiscus* adults, the gas stores contain less than 1% oxygen after 3–4 min. submergence at 16–17° C., while in *Notonecta* the oxygen is reduced to 5·3% after 2 min. and to 1·6% after 4 min. at the same temperature (Ege, 1915). In *Anisops*, on the other hand, the gas stores

may still contain about 3 % oxygen (20 mm. Hg) towards the end of a dive (3–4 min.) at 25° C. For the same reasons it may be argued that the loss of oxygen by *Anisops* in oxygen-poor waters will be small. These conclusions are supported by the observation that dives performed in oxygen-deficient water and in well-aerated water have approximately the same duration (Miller, 1964*b*). In conclusion therefore it seems that during a normal dive, *Anisops pellucens* may derive as much as 75 % of the oxygen consumed from its haemoglobin, a further 25 % from its gas stores and a negligible amount from the water.

SUMMARY

1. The percentage saturation of the haemoglobin of the intact water bug, *Anisops pellucens*, in equilibrium with various ambient oxygen tensions has been determined. From this an approximate dissociation curve for the pigment and a P_{50} value of 28 mm. Hg at 24° C. have been obtained.

2. *Anisops* haemoglobin shows no Bohr effect, but appears to be very temperature-sensitive.

3. During free dives the haemoglobin is regularly de-oxygenated; the oxygen so derived probably helps to maintain the bug in neutral buoyancy for much of each dive.

4. Carbon monoxide has been used to inactivate the haemoglobin, after which dives are reduced to about one-fifth of their normal duration and the phase of neutral buoyancy is abolished.

5. Much of the oxygen which leaves the pigment probably diffuses into the ventral airstore and then re-enters the tracheal system via the thoracic spiracles. External 'pumping' movements may serve to speed this gas migration. It is argued that physical gill action plays a negligible part in the normal respiration of this species.

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