11–2 RESPIRATORY PIGMENTS

We can only account for the distribution of oxygen carriers in the animal kingdom if we assume that these compounds have been independently evolved in many different lines, for more than one type of compound has been employed, and the distribution of these follows no simple phylogenetic plan. The most widespread of these substances is the group of compounds called haemoglobins; these have certainly evolved quite independently in unrelated groups, doubtless as a consequence of the capacity of living organisms for synthesizing the compounds known as porphyrins, which may be regarded theoretically as derivatives of a parent compound called porphin (Fig. 11–1). This substance, known only from laboratory synthesis, consists of four pyrrole rings linked by four methane bridges into a cyclic system which is evidently of great stability, for porphyrins of biological origin occur in mineral deposits such as coal and oil. They are widely distributed in living organisms, in bacteria and plants as well as in animals. In fact, the capacity for synthesizing them is universal in aerobic organisms, with the exception of certain bacteria, and they are probably another example of a molecular pattern that appeared very early in the history of evolution.

A particularly important property of porphyrins is their ability to associate with

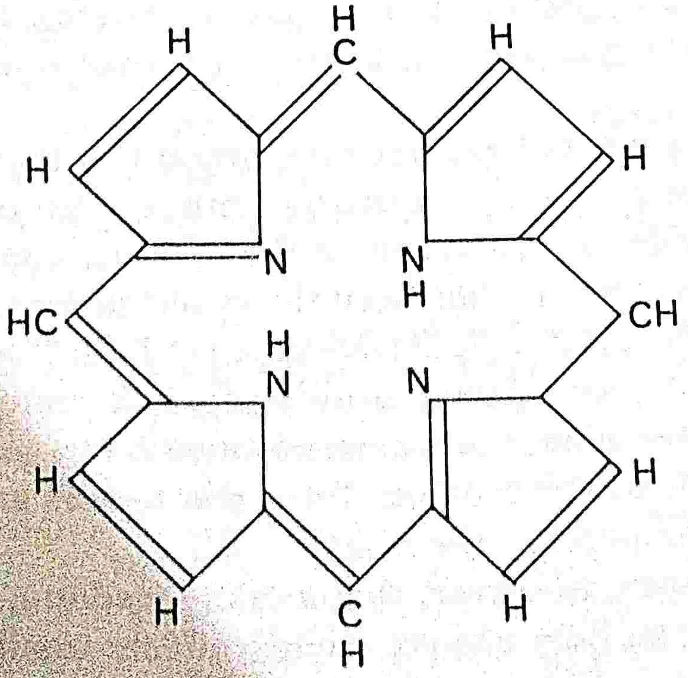


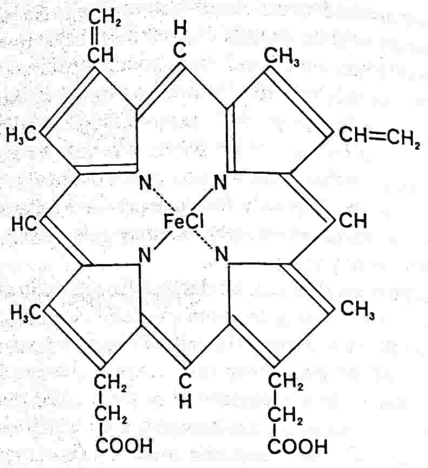
Fig. 11-1. Porphin.

metals to form coordinated compounds known as metallo-porphyrins. These compounds serve as the prosthetic groups of proteins that have a wide range of catalytic functions in the cell. Thus iron porphyrins are the prosthetic groups of the peroxidases that are found largely in plant tissues, and also of the catalases of bacteria and animal tissues, while chlorophyll is a magnesium-porphyrin complex (Sec. 1–3). Furthermore, the cytochromes, which we have seen to be essential components of intracellular oxidation mechanisms, are composed of proteins having iron porphyrins as their prosthetic groups. Probably the capacity for synthesising this type of molecule led to the evolution of adaptively valuable compounds, with the consequent development of certain oxygen carriers.

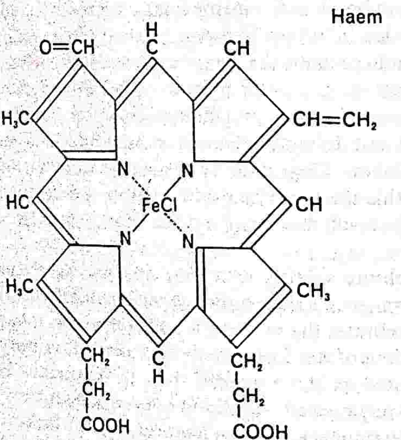
One of the porphyrins that can be derived from porphin is protoporphyrin. The addition to this of one ferrous iron atom produces ferrous protoporphyrin (haem, Fig. 11–2), in which the iron atom is joined by four of its coordination bonds to the four nitrogen atoms of the protoporphyrin. One of the remaining six coordination bonds can then be joined to a molecule of protein called globin. The result is the formation of the compounds known as haemoglobins, which are the best known of all of the oxygen carriers. The haemoglobin molecule can carry an oxygen molecule attached to the remaining coordination bond, with the iron remaining in the ferrous state (Fig. 11–2); this is of peculiar value in oxygen transport. The oxygen is readily taken up at the respiratory surface, and is equally readily given up within the tissues at regions of low oxygen concentration; this latter process, called dissociation, involves the replacement of a molecule of oxygen by a molecule of water. The two forms of the compound, oxygenated and deoxygenated, are known respectively as oxyhaemoglobin and deoxyhaemoglobin. They must be distinguished from the oxidized form, methaemoglobin; in this the iron is in the ferric form, and the sixth bond carries a hydroxyl group, with the result that the molecule is no longer available as an oxygen carrier.

Haemoglobins occur in all vertebrate animals, with some very rare exceptions (the leptocephalus larva of the eel is one), so that they can be regarded as a biochemical characteristic of the group. In invertebrates the situation is quite otherwise. It has been known since the early investigations of Ray Lankester in the nineteenth century that haemoglobin is widely distributed in these animals, but its appearance is curiously sporadic, and of no obvious phylogenetic significance. As suggested above, this is most readily explained as a consequence of the widespread capacity for synthesizing protoporphyrin. Conceivably haemoglobin could have arisen because of changes in the cytochromes resulting from mutation. Cytochromes, however, depend for their functioning upon oxidation, with an associated change in the valence state of the iron from the ferrous to the ferric form. Thus, as Munro Fox and Vevers remark, it is probably easier to imagine haemoglobins arising from the chance association of protoporphyrin with various globins.

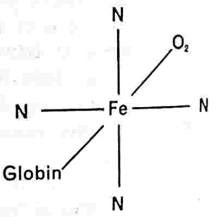
Among the invertebrates haemoglobin is particularly characteristic of the Annelida and the entomostracan Crustacea, where it is typically dissolved in the blood fluid, although it may also be present in the tissues. It occurs only rarely in other arthropods, the blood of the midge larva, *Chironomus*, providing one example. In molluscs its occurrence is sporadic; when present it is usually in the tissues, but it is dissolved in the blood of one pulmonate, *Planorbis*, and it occurs in blood corpuscles in a few bivalves. It has been identified also in animals as diverse as *Para-*



Haem



Chlorocruorohaem



Linkages of ferrous iron in oxyhaemoglobin

Fig. 11-2. Haem and related compounds.

mecium, trematodes, nemertines, *Ascaris*, and *Phoronis*. Finally, a closely similar substance (leghaemoglobin) is found in the root nodules of Leguminosae, where it results from the interaction between the plant itself and its symbiotic micro-organism, *Rhizobium*; neither of these can produce it unaided.

Oxyhaemoglobin and deoxyhaemoglobin are respectively red and purple in colour, and are characterized by well-defined absorption bands in the visible spectrum. Oxyhaemoglobin has alpha and beta bands in the yellow and green regions (the alpha nearer the red end) and a larger gamma band in the violet region. Deoxyhaemoglobin has the gamma band, although at a slightly different position, together with only one other band, lying in the green region. The positions of these bands vary in haemoglobins from different sources, which means, of course, that there must be many different haemoglobins. Indeed, spectroscopic characteristics are not the only differences between them. They are distinguishable also by physical properties, such as their isoelectric points and their amino acid composition, and these are reflected in differences in the kinetics and equilibria of their interactions with oxygen. These characteristics arise solely from differences in the globin portion of their molecules, the haem portion being always identical. This can be demonstrated by taking advantage of the capacity of pyridine for displacing the globin and combining with the haem to form a pyridine–haem complex. The spectroscopic properties of this complex are always precisely the same, irrespective of the properties of the haemoglobin from which it is derived.

All haemoglobins consist of unit molecules, each with one haem and its globin, and having a molecular weight of 17,000–18,000. In general, the invertebrate haemoglobins differ from the vertebrate ones in having less histidine and lysine, and more arginine and cystine, but they also differ among themselves in the number of units that they contain. These range from two in the larva of *Chironomus* (m.w. 31,400), for example, to several hundred in *Arenicola* (m.w. 3 million). This variability has doubtless helped to provide for the adaptive molecular evolution of haemoglobins that are particularly suited for use in different types of environment. There are, however, other differences to be found in respiratory pigments, showing that substances that are similar in principle in their oxygen-transporting properties may yet be very different in their molecular structure.

One example of these is chlorocruorin, a green respiratory pigment found only in four families of the Polychaeta: the Ampharetidae, Chlorhaemidae, Sabellidae, and Serpulidae. It is closely related chemically to haemoglobin (Fig. 11–2). The protein is different, as is to be expected, but the iron porphyrin of its prosthetic group is a haem which differs only in having one of the two vinyl groups ($-CH{=}CH_2$) substituted by a formyl (aldehyde, $-CHO$) group. Its molecular weight, however, is extremely high, amounting to about 3 million, and it contains some 190 iron atoms in its complex molecule. Chlorocruorin is described as dichroic, for it has two colours which are dependent on its concentration, green in dilute solutions and red in concentrated ones. Worms with this pigment are not necessarily green, however, for the colour may be masked by other pigments, as it is, for example, in the tentacles of serpulids. More confusing is the fact that closely related species may differ in the presence or absence of the pigment, as in the genus *Spirorbis*. One species, *S. borealis*, possesses chlorocruorin; another, *S. corrugatus*, has haemoglobin; a third, *S. militaris*, has no respiratory pigment at all.

The close similarity of chlorocruorin to haemoglobin makes it likely enough that chlorocruorin could evolve from haemoglobin by genetic mutation, and that it might well have arisen independently in different species. Thus the presence of this pigment is not necessarily evidence of the close phylogenetic relationship of the groups concerned. The need for caution in making such interpretations is shown by the presence of chlorocruorohaem, which is the prosthetic group of chlorocruorin, in two starfish, *Luidia* and *Astropecten*; these animals have not, however, developed the respiratory pigment itself.

The devious pathways of biochemical evolution are further illustrated by the haemerythrins. These are reddish-violet iron-containing respiratory pigments that are known only in the sipunculids (including *Sipunculus* and *Phascolosoma*), the polychaete *Magelona*, the priapulids *Halicryptus* and *Priapulus*, and the brachiopod *Lingula*. Haemerythrin is thus a rare pigment, and, apart from the fact that it has been identified in all sipunculids examined, its distribution is sporadic and without any phylogenetic significance. The sipunculids are possibly related to annelids, but the priapulids are non-coelomate forms, while the brachiopods are perhaps related through the structure of their lophophore to *Phoronis* and the Ectoprocta. There are other distinguishing features of this pigment. For one thing, it is always present in cells, which are usually in the coelomic fluid; in *Magelona*, however, they are true blood corpuscles, this animal being the only polychaete to carry a respiratory pigment in this particular way. This intracellular location of haemerythrin is doubtless correlated with its molecular weight (66,000 in *Sipunculus*), which is low in comparison with that of most invertebrate respiratory pigments, and more comparable with that of vertebrate haemoglobin (68,000). Retention of respiratory pigments of low molecular weight within cells is thought to be an adaptation preventing their loss through the excretory system. Another feature of haemerythrin is that, despite its oxygen-carrying capacity and its red colour, it is not closely related to haemoglobin. Its spectrum shows no strong absorption bands, and in molecular structure it is a protein, with a high percentage of iron in its molecule, but with no associated porphyrin, the iron being attached directly to the protein. Thus in haemoglobin and haemerythrin we find a similar result achieved by different chemical means.

Haemerythrin is misleadingly named, and so also is haemocyanin, another respiratory pigment in which the prosthetic group is not a haem, and in which the metal is probably attached directly to the protein. Here, however, the metal is copper instead of iron. In this respect haemocyanin differs from all of the previously mentioned respiratory pigments, and shows some relationship to the copper-containing phenol oxidases, a relationship analogous to that of the haemoglobins to the iron-containing oxidases and peroxidases. The oxygen combines with two atoms of copper in haemocyanin, as compared with the one iron atom of the haemoglobin, and in so doing produces a blue compound, a deoxygenated form of the pigment being colourless. The molecular weights are considerable—units with two copper atoms range from 50,000 to 74,000, and are associated into complex molecules with molecular weights as high as 6,650,000 in *Helix pomatia*. Not surprisingly there is variation here, as with the haemoglobins, in the properties of the pigment.

Haemocyanin, like chlorocruorin, occurs only in solution, in this instance in the haemolymph of certain arthropods and molluscs, where its distribution shows a certain phylogenetic pattern. It is the only respiratory pigment of malacostracan Crustacea, occurring in the decapods and the stomatopods. It is found in no other crustaceans, nor does it occur at all in insects, but it appears in some chelicerates: *Limulus*, *Euscorpius*, and spiders. As regards molluscs, it is found in chitons, in cephalopods, and in many gastropods, including particularly the prosobranchs and the pulmonates, but it has not yet been identified in the bivalves. Molluscan haemocyanins have molecular weights of several million, while those of crustaceans are of the order of several hundred thousand; the difference doubtless reflects the independent origin of these substances in the two groups.

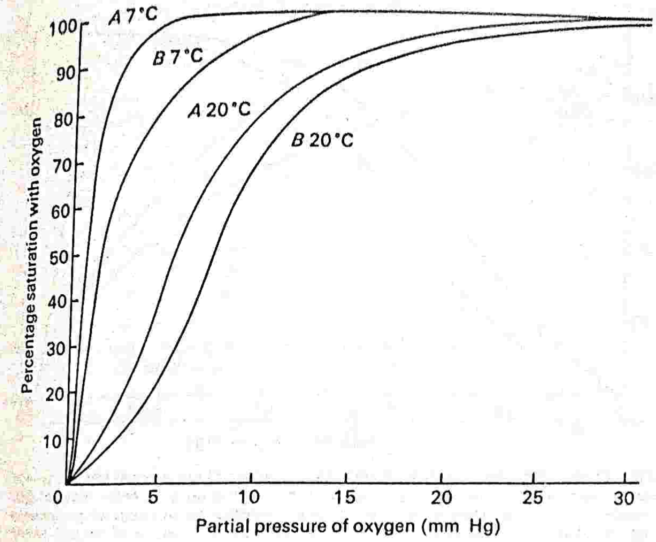


Fig. 11-3. Oxygen dissociation curves for the haemoglobins of two earthworms, *Allolobophora terrestris* (*A*) and *Lumbricus terrestris* (*B*) at the temperatures shown. These are high oxygen affinity pigments. From Haughton *et al.*, 1958. *J. exp. Biol.* 35, 360–368.

The wide distribution of respiratory pigments, and their occurrence in such different groups, is sufficient indication that they must confer some respiratory advantages. Yet it needs to be emphasized that, as in the case of *Spirorbis*, one species may exist without any of these pigments, while related species possess them. Indeed, it is not always easy to decide just what their function is in certain species. As we have already suggested, the molecules of these pigments seem well suited for undergoing adaptive modification. Yet we must judge their function not only from the properties of the particular pigment, but also with an understanding of the conditions of life of the species concerned.

In general, respiratory pigments function either as oxygen carriers, or as oxygen stores, providing reserves to be used at times of shortage. The former function may reasonably be attributed to those pigments that are present within the blood stream, but where they are present in other tissues (as, for example, in the muscles of *Arenicola*) they are more probably serving for oxygen storage. However, storage is not sharply separable from transport, for the tissue pigments, by combining with the oxygen arriving in the blood stream, must accelerate the passage of oxygen into the tissues. This is likely to be particularly important in organs that carry out rhythmical bursts of activity; it is significant, therefore, that there is evidence of haemoglobin being particularly abundant in the muscles of gizzards and radulae. Moreover, its distribution may be adapted to the mode of feeding, as it appears to be in *Aplysia*,

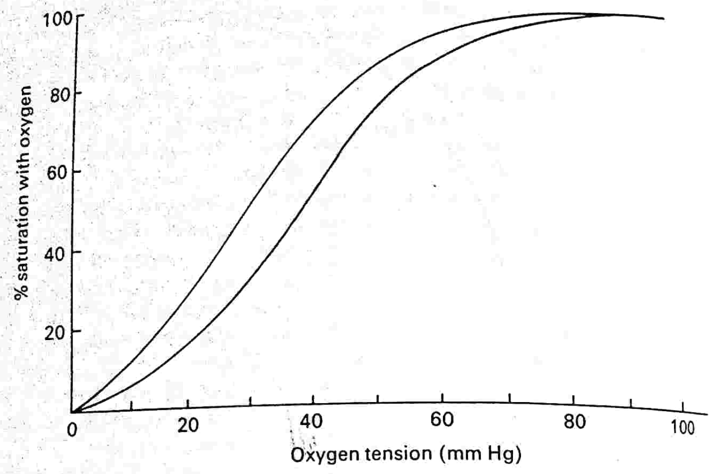


Fig. 11-4. The Bohr effect, illustrated by a pigment of low oxygen affinity, typical of man and other mammals. The curve is shifted to the right by an increase of CO_2 tension in the blood stream; such an increase, which may be expected to occur in regions of high metabolic activity, will thus facilitate the release of oxygen to the tissues.

which feeds continuously, and the related *Navanax inermis*, which feeds sporadically. The former has muscle haemoglobin (myoglobin) in its buccal musculature, whereas the latter lacks it. It is often supposed that storage is also the main function of the respiratory pigment of the body cavities, such as the coelomic haemoglobins of some annelids and the coelomic haemerythrins of sipunculids. This may be so, but we have noted that the use of such fluids in hydrostatic skeletons results in a good deal of movement in them, and certainly brings them into close functional relationship with contractile tissues; some transport function cannot, therefore, be excluded in such instances.

One key factor in the mode of functioning of these pigments is the relationship between the amount of oxygen that they can take up and the pressure of oxygen (oxygen tension) to which they are exposed. This relationship is expressed in an oxygen dissociation curve (or oxygen equilibrium curve), which is obtained by exposing the blood to oxygen at a series of different tensions. Examples of such curves are seen in Fig. 11–3, which shows the dissociation curves for two species of earthworms. These have haemoglobins of high oxygen affinity, which are well saturated at a wide range of environmental oxygen tensions. Their low unloading tensions imply that oxygen tensions are correspondingly low in the tissues. This, it is suggested, may be due to the lack of respiratory organs, combined with a body surface that has a high resistance to diffusion.

It will be apparent that a useful index of the mode of functioning of these pigments is given by determining the partial pressure of oxygen at which they are half-saturated with oxygen. This value, expressed as p_{50}, varies with temperature, lower temperatures shifting the curves to the left, and vice versa (Fig. 11–3). It is also

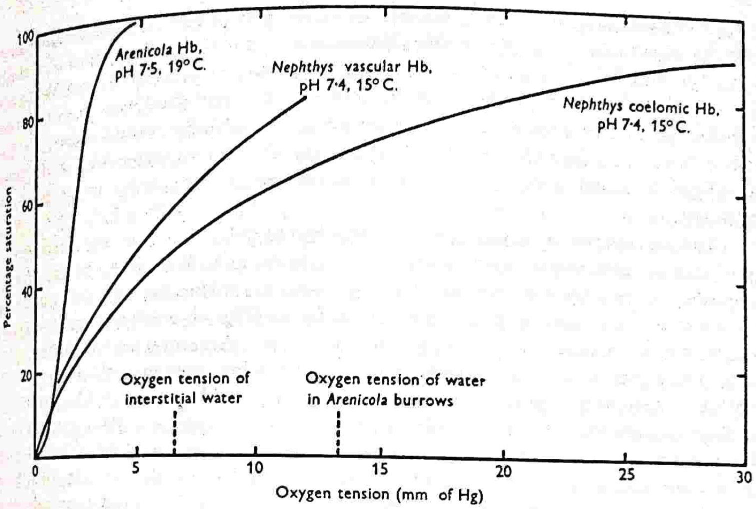


Fig. 11-5. Comparison of the oxygen dissociation curves of the vascular and coelomic haemoglobins of *Nephtys hombergii* at pH 7.4 and 15°C and of the haemoglobin of *Arenicola marina* at pH 7.5 and 19°C. The levels of interstitial oxygen tension and of oxygen tension in the residual water of exposed *Arenicola* burrows are indicated by vertical lines above the abscissa. From Jones, 1955. *J. exp. Biol.*, 32, 110-125.

affected by acidity. If the pH decreases, so does the oxygen affinity, and the curve is moved to the right (Fig. 11–4). An increase in carbon dioxide tension will have this effect (the Bohr effect), and since this tension will be high where tissues are active, the result is to facilitate the release of oxygen to the tissues that most need it. A reversed Bohr effect is sometimes found, the dissociation curve shifting to the left with a rise in pH.

It has often been suggested that a low oxygen affinity indicates an oxygen transport function for the pigment, but that a high affinity may indicate a storage function, allowing oxygen to become available in conditions in which transport has become impossible. This, however, is an oversimplification, as may be illustrated by reference to *Arenicola*. The oxygen affinity of the haemoglobin of this animal is high (Fig. 11–5), with a p_{50} ranging from 2.0 to 8.3 mm, while its oxygen-carrying capacity is exceptionally high for an invertebrate. Even so, however, this capacity is low in relation to the known metabolic rate of the animal. Even if the pigment were functioning solely as a store, the oxygen would only be sufficient for about 21 min of activity. Admittedly even as small a store as this could be of value in the intervals between the rhythmic irrigation movements which, as we shall see later (Sec. 11–3), the worms carry out in their burrows, and which provide a regular replenishment of the oxygen supply. However, it is probable that the pigment does, in fact, provide a mechanism for transporting oxygen. The oxygen tension of the water in the sand around the burrow at low tide will contain an oxygen concentration equivalent to about 6.7 mm of mercury. Reference to Fig. 11–5 shows that the pigment can continue to take up oxygen at even lower tensions than this, so that it is well able to transport it to the tissues and release it to the haemoglobin of the muscle. We shall see that the respiration of *Arenicola* is further aided by the animal's ability to draw oxygen into its burrow when the tide is out; presumably, therefore, it is not dependent for long periods upon stored oxygen. We may conclude that the transport capacity of the haemoglobin of *Arenicola* is well adapted to the needs of an animal which experiences a wide range of oxygen tensions in its burrow. At the same time, some storage function is not excluded.

Another burrowing polychaete, *Nephtys hombergii*, presents an interesting contrast to *Arenicola*. It has haemoglobin in the coelom as well as in the blood, the p_{50} values being, respectively, 5.5 and 7.5 mm. These are within the range of values for *Arenicola*, but the shape of the curve is very different (Fig. 11–5), showing that oxygen is released over a much wider range of tensions. This, therefore, is a low-affinity pigment. The difference can be ascribed to *Nephtys* moving vigorously through the substratum in temporary burrows that collapse when the tide is out. Unlike *Arenicola*, therefore, it has no substantial store of environmental oxygen, while it can get little benefit from the sparse interstitial water, for the oxygen tension of this is so low as to be virtually unusable. A high-affinity pigment would be of little value for transport when the tide is in. When it is out, the animal must reduce activity, and its metabolism then is probably largely anaerobic. Where so many invertebrates differ from mammals is in their ability to maintain metabolism at very low internal oxygen tensions. This is probably the situation in *Arenicola*, and it is probably true also of *Tubifex* and earthworms, which can continue their metabolism even when their haemoglobin is completely immobilized by the presence of carbon monoxide.