LETTERS TO THE EDITORS

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Oxygen and Hæm in Invertebrates

THE quantity of hæmoglobin in solution in the blood of *Daphnia* is inversely proportional to the dissolved oxygen content of the water in which the animals live[1,2]. In the course of a few days *Daphnia* can change in tint from almost colourless to red, or the reverse, by the synthesis or breakdown of hæmoglobin. This occurs, too, in other branchiopod Crustacea (for example, Estheria, Apus, *Artemia*). Since a gain of blood-hæmoglobin with fall of external oxygen pressure is known also in man at high altitudes and fishes in poorly aerated water, one may ask if it occurs in all animals with hæmoglobin.

One of the most familiar invertebrate animals with abundant hæmoglobin in solution in the blood is the larva of the midge *Chironomus*. It lives in a mucous tube embedded in mud at the bottom of ponds. Keeping larvæ with well-aerated and poorly aerated water above the mud was found to have no influence on their hæmoglobin content. This may have been because the larvæ control the oxygen content of the water in their tubes; they draw in water by undulatory body movements, which are intermittent, the intervals becoming longer when the water is well aerated[3]. An attempt was therefore made to keep the larvæ without mud, but it failed because larvæ kept thus die in a few days. Young larvæ, however, freshly hatched from the egg, can live without mud. Fed on *Chlorella*, they grew and made mucous tubes, but frequently left them to swim in the water. These young larvæ formed more hæmoglobin in poorly than in well-aerated water. The partially and fully grown larvæ of another midge, *Anatopynia varia* (Fabr.), which make no tubes, also had more hæmoglobin in poorly aerated water.

The most familiar mollusc with hæmoglobin in solution in its blood is the ramshorn pond snail, *Planorbis corneus* (L.). Keeping adults for a month in water of high or low oxygen content had no effect on the redness of the blood; but newly hatched young reared in poorly aerated water became, in less than a month, redder than those grown in well-aerated water. It was not, however, found possible to influence the hæmoglobin content in the blood of annelid worms by changes in external oxygen concentration.

In the vertebrates hæmoglobin is found in muscle cells as well as in the blood. In invertebrates hæmoglobin occurs not only in blood and muscle, but also in nerve, fat and tracheal cells, cœlomic corpuscles, ova, parenchyma of rhabdocœles and bodies of Protozoa[4]. In mammals it is uncertain whether there is an increase in muscle hæmoglobin at high altitudes[5], but muscular exercise has this effect[6], owing perhaps to local oxygen deficiency. It was found that hæmoglobin increases and decreases in concentration in muscles and nerve ganglia of cladoceran and conchostran Crustacea just as it does in the blood, in response to paucity or sufficiency of oxygen in the environment. There was, however, no such effect of oxygen on the hæmoglobin content of pharyngeal muscles in the pond-snail *Physa*, or of the parenchyma in the rhabdocœle worm *Phaenocora*.

Thus, among the invertebrates, in some but not all instances, hæmoglobin of blood or tissue cells fluctuates in quantity inversely as the oxygen pressure in the environment. Finally, cytochrome, detectable with the microspectroscope in muscles of *Daphnia* and Conchostraca, increases and decreases in concentration, like the hæmoglobins of blood, muscle and nerve, as the oxygen in the water decreases or increases. In mammals, too, an increase in the quantity of cytochrome in muscle occurs in response to a low atmospheric pressure[7]. It is curious, however, that the increase of cytochrome in mammals and Crustacea in response to diminished oxygen is the reverse of what occurs in baker's yeast, where the synthesis of cytochromes *a*, *b* and *c* is stimulated by air and inhibited by its absence[8,9].

A full account of this work will be published in the *Proceedings of the Royal Society*.

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Passage of Glucose and Glycerol across the Red Cell Membrane

DURING recent years, increasing evidence has accumulated indicating that the passage of glucose across the human red cell membrane and of glycerol across the human and rabbit membranes is not a process of simple diffusion. The results of Wilbrandt and Rosenberg[1], Lefevre[2] and Widdas[3] show that the kinetics are not those of a simple diffusion process, and the effect of enzyme inhibitors indicates that at least specific adsorption sites are involved. The work of Lefevre[4] showed the inhibiting action of —SH agents; for example, *p*-chloromercuribenzoates, iodine and mercuric ions on glucose and glycerol transfer, the work of Jacobs and Corson[5] showed the inhibition of glycerol transfer by copper, and Wilbrandt[6] showed that glucose penetration is inhibited by lachrymators whereas glycerol remains unaffected. The passage of both glycerol and glucose is inhibited by phloridzin[4].

Though at first sight it would appear that the process is an active transfer involving an enzymic reaction providing energy other than thermal agitation, this is not necessarily so; and the process is perhaps better regarded as facilitated diffusion[7]. In this case the process differs from simple diffusion in being restricted by both structural and steric factors, and energy may be required for maintenance of structural units. It has been pointed out[7] that the distinction between active transport and facilitated diffusion may be one of degree only.

The present investigation was an attempt to study more closely the relationship between enzyme inhibition and the inhibition of facilitated diffusion by using irreversible inhibitors of the latter and showing strict parallelism to enzyme inhibition. The irreversible inhibitor chosen was 2–4 dinitrofluorobenzene. This substance was developed as a reagent for protein end-groups by Sanger[8] and also as a cytochemical agent by