ON THE OCCURRENCE OF HAEMOGLOBIN AND OF ERYTHROCYTES IN THE PERIVISCERAL FLUID OF A HOLOTHURIAN

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On examining specimens of *Cucumaria frauenfeldi*, it was found by one of us that a red fluid exuded from the body cavity when punctured. *C. frauenfeldi* (Ludwig), kindly identified for us by Dr Barnard of the South African Museum, is a common representative of the Holothuroidea in the littoral fauna of the Cape Peninsula, especially abundant in rock pools at Sea Point, near Cape Town. It is dark brown in colour and about $1\frac{1}{2}$ to 3 inches in length. On cutting through the integument, a densely coloured fluid of a port wine red or scarlet tint exudes. A few seconds after shedding the pigment is seen to be concentrated in flocculent masses which rapidly sediment leaving the supernatant liquor colourless. It can be dispersed again by vigorous shaking, and if then transferred to a test-tube connected with a pump capable of giving a high vacuum, it changes colour from scarlet to a reddish purple on removal of air. On readmitting the latter, and shaking once more, the scarlet colour reappears, thus indicating that the colouring matter itself is a reversibly oxidisable pigment, in all probability one of the family of haemoglobins, a conclusion confirmed, as will be seen later, by spectroscopic examination.

As haemoglobin has not been reported to occur in any Holothurian to the best of our knowledge, it seemed worthy of closer study, and an examination of the flocculent masses of pigmented substance, which sediment from the shed contents of the body cavity of *C. frauenfeldi* on standing, revealed a noteworthy and unexpected feature of the occurrence. This sediment proved to be exclusively composed of agglutinated masses of cells of a yellowish colour under microscopic observation; and the entire pigment contained in the fluid appeared to be concentrated within them. In short, the fluid which exudes from the perivisceral cavity of this Holothurian contains *erythrocytes* in great abundance. These erythrocytes are of fixed shape, viewed from one aspect more or less circular, tending to be ovoid or even somewhat fusiform in outline, there being some variability of shape *inter se*. In the plane at right angles they are seen to be thin discs, slightly bi-convex in contour. They are nucleated; but the nuclei are relatively small in relation to the cell as a whole, as compared with those of Vertebrate red cells. About the longest axes they measure approximately 30μ, the nucleus being about 3μ in diameter. The cytoplasm is not granular like that of the less abundant amoebocytes which occur with them. The fact that the pigment is exclusively contained in

them is easily demonstrated by the fact that on agglutination or sedimentation the serum is totally colourless. After centrifuging off the corpuscles and washing them repeatedly in sea water, they retain the pigment. They are readily laked by diluting the perivisceral fluid with distilled water containing a small quantity of ammonium oxalate.

A question that will at once be asked is whether these cells are found *in situ* in the perivisceral coelom or in the lacunar haemal canals, which are well developed in the Holothuroidea. On examining the organs, especially around the gut where the poorly developed vascular system of these animals is most in evidence, no sign of red vessels can be detected immediately after the fluid has been allowed to gush from the body cavity. Moreover in the freshly shed exudate the suspension of red cells is so dense as to leave little doubt that its source is in the main at least the perivisceral cavity. Some idea of the quantity of haemoglobin may be gained from the statement that 2–3 c.c. of fluid from a single individual were diluted 100 times for spectroscopic examination.

Superficial examination of the spectrum of the scarlet pigment in the freshly laked blood showed that it had the two characteristic absorption bands in the green typical of the haemoglobins; and that the reduced pigment has a single band overlapping the interspace between the α and β bands of the oxidised form. A carboxy derivative was prepared in the usual way, but the attempts to form methaemoglobin by the ferricyanide method and to prepare the acid and alkaline haematins in the manner prescribed for the haemoglobins of mammals proved inefficacious.

Two circumstances are specially worthy of notice in this connection: (i) bubbling of H_2S through a solution of Holothurian haemoglobin did not yield a compound analogous to the sulph-haemoglobins; (ii) though ammonium sulphide readily reduces the pigment, the addition of solid $Na_2S_2O_3$ either with or without Na_2CO_3 had no effect. The spectrometric axes of the carboxy, oxy and reduced pigments of *C. frauenfeldi* are compared with those of *Arenicola*, and of mammalian haemoglobin in the accompanying table which gives the wave-length corresponding to the middle point of the bands. The data are taken from papers by Barcroft and by Fox.

	Arenicola	Man	Horse (Fox)	Cucumaria
Oxy haemoglobin	α 575 β 540	α 576 —	α 578 β 543	α 579 β 543
Reduced haemoglobin	555	—	556	558
Carboxy haemoglobin	α 570 —	α 570 —	— —	α 573 β 538

In view of the correlation between the position of the absorption bands and affinity for O_2 and CO, as shown by the work of Barcroft, there is one further fact that is worthy of mention. Barcroft and Barcroft (1924) found that the unloading tension of the oxidised haemoglobin of *Arenicola* is so low that the pigment

cannot function in ordinary conditions as a means of transport of oxygen. In bleeding *C. frauenfeldi*, it was noticeable that in most cases, as stated earlier, the freshly shed perivisceral exudate was of a reddish purple tint, becoming scarlet on standing. From this it may be concluded that the unloading tension of Holothurian haemoglobin is not so low as to prevent it from giving up its oxygen to the tissues in normal conditions. This might be anticipated from Barcroft's rule, since the α band is shifted in the case of *Cucumaria*, as compared with mammalian haemoglobin, towards the red end and not towards the violet end, as in the case of Arenicolan haemoglobin.

Though in general haemoglobin, when it occurs in the tissue fluids or blood of invertebrate animals, is found dissolved in the plasma, in one other invertebrate at least, namely the mollusc Solen, Lankester (1872) reports the existence of red cells. Echinochrome, the red pigment of the test and eggs of *Arbacia* and some other Echinoidea (*Echinus sphaera*, *E. esculentus*, *Amphidotus* and *Strongylocentrotus* sp.), is also found in cells of the perivisceral fluid (elaeocytes); but echinochrome is not a true respiratory pigment, since it has recently been shown by Cannan that it does not combine reversibly with molecular oxygen *in vitro*. When reduced with strong reducing agents, it becomes colourless—not purple like haemoglobin. The red cells of *C. frauenfeldi* are therefore essentially different both physiologically and chemically from the cells carrying echinochrome in the perivisceral fluid of sea urchins.

REFERENCES.

BARCROFT (1925). *Physiol. Rev.* **5**.
CANNAN (1927). *Biochem. Journ.* **21**.
FOX (1924). *Proc. Camb. Phil. Soc.* **1**.
LANKESTER (1872). *Proc. Roy. Soc.* **21**.