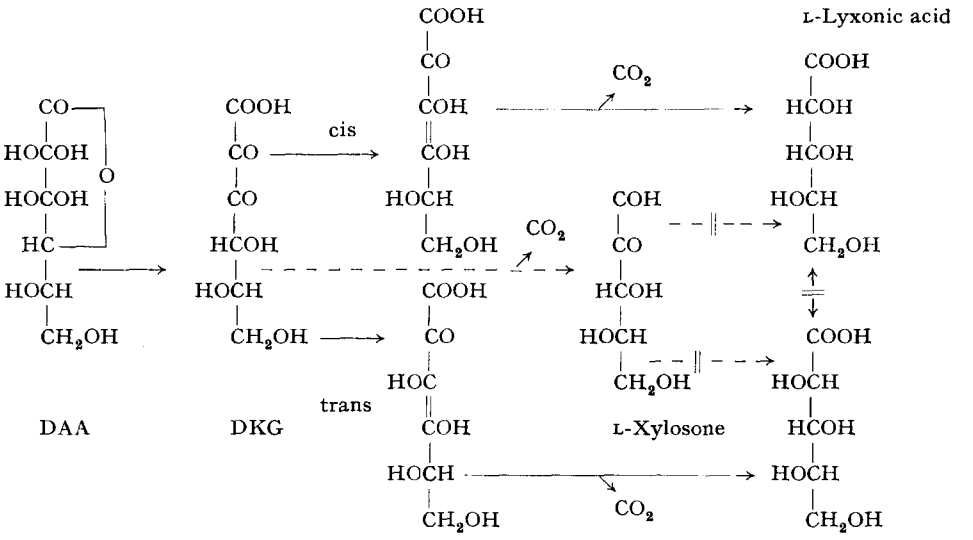
disodium DKG was used as a substrate, contrary to DKG which showed a 1-min lag. The sharp decrease in activity in acid conditions (pH 6.2) and the high apparent K_m ($1.5 \times 10^{-2} M$) also supported the presence of a enediol intermediate.



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Cytochrome-like haemoproteins in the gut fluids of molluscs

Helicorubin, a haemoprotein of unknown physiological function, has been found in the gut fluids of certain molluscs, polychaetes and crustaceans[1–3], and some of its physical and biochemical properties have recently been characterized with purified preparations[4,5]. Except for erythrocruorin and chlorocruorin occurring in the blood of invertebrates, helicorubin is the only haemoprotein found extracellular in high concentrations.

Abbreviation: DEAE, diethylaminoethyl-.

During the studies on cytochromes of invertebrates[6,7], the author discovered two cytochrome-like haemoproteins, one in the gut fluids of garden snails, *Euhadra amaliae* and *E. sandai*, and the other in those of fresh-water mussels, *Hyriopsis schlegelii* and *Cristaria plicata* (Table I). They are described here under the name of "Enterochrome 556" for the gut haemoprotein of garden snails and "Enterochrome 566" for that of fresh-water mussels.

TABLE I

ABSORPTION MAXIMA OF PURIFIED HAEMOPROTEINS AND CONCENTRATIONS IN GUT FLUIDS

Haemoprotein	*Absorption bands of reduced form*			*Animal*	*Concentration*** (μM)*
	α (mμ)	*β (mμ)*	*Soret (mμ)*		
				Garden snail	
Enterochrome 556	556	526	424 (412)*	*Euhadra amaliae*	58–83
				E. sandai	—
Helicorubin**	561.5	530	427 (415.5)*	*Helix pomatia*	
				Fresh-water mussel	
Enterochrome 566	566	530	430 (416)*	*Cristaria plicata*	14–35
				Hyriopsis schlegelii	18–21

* Oxidized form.
** J. KEILIN (1956)[4].
*** The haem concentration was calculated from difference spectra. The extinction coefficients were assumed to be equal to that of cytochrome *b* (ref. 8).

The collected gut fluid from the gastrointestinal tracts of starved garden snails given only a little water or from the stomach cavities of starved fresh-water mussels, was diluted 3-fold with water, and treated with a small amount of 0.5 *M* lead acetate. After removal of the excess of lead with a small amount of Amberlite CG-50 (sodium form), the solution was fractionated with $(NH_4)_2SO_4$. The fraction precipitating between 0.45 and 0.75 satn., was collected, dialyzed, and then applied on a DEAE-cellulose column equilibrated with 0.01 *M* phosphate buffer, pH 7.0, the elution being carried out with the same buffer. The denatured haemoproteins and brown pigments were strongly absorbed on the top of the column and not elutable by the buffer. The eluted fraction, after saturated with $(NH_4)_2SO_4$ at 0.75, was dialyzed against 0.001 *M* phosphate buffer, pH 7.0, then concentrated by the freeze-drying.

Both haemoprotein preparations thus obtained were clear orange-red solutions. Figs. 1A and B show their visible absorption spectra. Enterochrome 566 had a shoulder at 560 mμ in addition to the α-band at 566 mμ. The prosthetic groups of both enterochromes can be easily split off by treating them with acetone containing 1% HCl, and the acid haematins thus separated were soluble in ether. The reduced bands of the alkaline pyridine haemochromogen prepared from enterochrome 566 lie at 557 mμ (α), 526 mμ (β) and 418 mμ (Soret). However, those of enterochrome 556 lie at 553 mμ (α), 521 mμ (β) and 415 mμ (Soret). The oxidation–reduction potentials of the two haemoproteins, determined with the aid of 2,6-dichlorophenol-indophenol and toluylene blue at 30°, were found to be nearly equal, *viz.* + 0.14 V at pH 7.8 and + 0.22 V at pH 6.4. Both of the enterochromes migrated toward the cathode when they were subjected to paper electrophoresis (0.05 *M* phosphate buffer, pH 7.0) and in this respect resemble mammalian cytochrome *c*. The enterochrome

preparations showed no peroxidase activity when assayed in the guaiacol test[9]. These haemoproteins did not react with CO and cyanide. They were reducible by ascorbate as readily as by $Na_2S_2O_4$, but not reducible by succinate or reduced diphosphopyridine nucleotide.

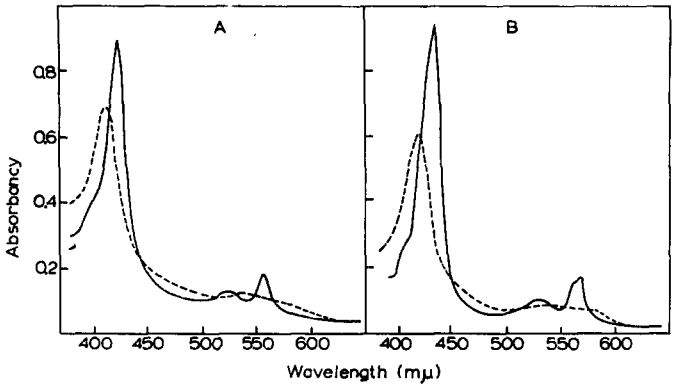

Fig. 1. Absorption spectra of gut haemoproteins in the oxidized form (dotted line) and after reduction with $Na_2S_2O_4$ (full line). A, enterochrome 556 from *Euhadra*; B, enterochrome 566 from *Hyriopsis*. Each haemoprotein was in 0.05 *M* phosphate buffer, pH 7.0.

In view of its absorption characteristics in the presence of pyridine and its ready splitting in acid acetone, the prosthetic group of enterochrome 566 may be regarded as protohaem. The haem moiety of enterochrome 556, on the other hand, seems to represent a new type of haem, in that it is easily split by acid acetone and has an α-peak at 553 mμ in its pyridine haemochromogen. Although enterochrome 556, in certain respects, is similar to cytochrome *h* extracted from the hepatopancreas of *Helix pomatia*[4], it is very different from cytochrome *h* in the following respects: mode of existence, position of the Soret absorption band and behavior on paper electrophoresis.

A full report of this work will be presented for publication shortly. The author wish to thank Prof. RYO SATO for his continual interest and advice.

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