whether or not the enzymes investigated by Slein and by us are identical.

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Cytochrome System in Oyster Tissues

Although some experiments with tissue extracts of the oyster have proved the presence of an oxidase very similar to the cytochrome oxidase of higher animals[1,2], it is still obscure whether the cytochrome system functions as terminal oxidase in the intact tissues of oysters, because even cytochrome has not been observed spectroscopically. Recently, Jodrey and Wilbur[2] suggested that the cytochrome system may not play a major part in the oxidative metabolism of the oyster mantle (*Crassostrea virginica*), since methylene blue ($1{\cdot}4 \times 10^{-5}$ *M*) did not reverse the cyanide inhibition of the tissue.

In the present investigation, absorption bands of the cytochromes were observed with the tissue homogenate of the oyster (*Crassostrea gigas*), when reduced with hydrosulphite, by applying the liquid-air method of Keilin and Hartree[3]. The absorption bands, however, were very faint compared with those of baker's yeast. The band corresponding to cytochrome *b* (560–565 mμ at room temp., 560 mμ at liquid-air temp.) was detected in all tissues examined, namely, gill, mantle, adductor muscle and digestive diverticula. However, the band *a* (around 600 mμ) was observed only in the gill and the mantle, and no trace of cytochrome *c* was detected. A very strong pyridine hæmochromogen band (550–560 mμ) was readily obtained with all tissues.

In a gas mixture of 90 per cent nitrogen and 10 per cent oxygen, the respiration of both the mantle and the gill was inhibited by about 50 per cent in the dark, and this inhibition was reversed almost completely by illumination with a 500-watt lamp (Table 1).

The respiration of both the mantle and the gill was also reduced by 0·001 *M* cyanide to about 18 per cent of the control value. In the presence of a nearly saturated solution of methylene blue in sea water (6×10^{-5} *M*), the inhibition was reversed to about 40 per cent; it was confirmed that a lower concentration of methylene blue (1×10^{-5} *M*) did not reverse the cyanide inhibition.

From these experiments it is evident that the respiration of the oyster tissues is largely dependent on metabolic pathways involving the cytochrome–cytochrome oxidase system. The fact that no band of cytochrome *c* was detected in any tissue examined, or of cytochrome *a* in certain tissues of the oyster, may be attributed to the low concentration.

Table 1

Tissue	Time (min.)	Oxygen uptake (μl.)*	
		90 per cent nitrogen + 10 per cent oxygen†	90 per cent carbon monoxide + 10 per cent oxygen
Mantle slices, 100 mgm. wet weight	0–40	31	11, dark
	40–80	29	26, light
	80–120	28	14, dark
	120–160	26	26, light
Gill pieces, 50 mgm.	0–50	34	18, dark; 34, light
	50–100	31	30, light; 17, dark

* The oxygen uptake was measured in Warburg manometers, with vessels about 9 ml. capacity, at 25° C. The vessels contained 1·5 ml. of 0·03 *M* glycine or glycylglycine-buffered sea water (*p*H 8·0) and 50 or 100 mgm. of tissue pieces.
† There was no difference between oxygen uptake in this mixture and in air.

Details of this work will be published elsewhere.

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Structure of Sperm- and Sei-Whale Insulins and their Breakdown by Whale Pepsin

As described in previous papers[1], our experimental results, using the method of Moore and Stein[2], have shown that the amino-acid composition of whale (sei-) insulin differs from that reported by Sanger *et al.*[3]. Furthermore, we have recently re-examined the amino-acid analyses of sperm- and sei-whale insulins (whole insulin and both glycyl (*A*) and phenylalanyl (*B*) chains of oxidized insulin, respectively). These experiments have confirmed that the amino-acid content of sperm insulin is similar to that obtained by Sanger *et al.*; but sei-whale insulin contains an alanine instead of an *iso*leucine residue in the *A* chain.

The amino-acid sequences in both sperm- and sei-whale insulins were then compared with the sequence reported by Sanger *et al.* The *B* chains were found to have the same sequence as Sanger's, but there were species differences in positions 8–10 of the *A* chain: in sperm insulin, Thr.Ser.Ileu (identical with Sanger), and in sei-whale insulin, Ala.Ser.Thr.

Crystalline sperm- and sei-whale insulins[4], and crystalline sei-whale pepsin[5], which we have previously prepared and described, were recrystallized 4 and 7 times respectively. Crystalline swine pepsin was obtained from the Worthington Chemical Laboratory and recrystallized four times. Pepsin was used in a glycerol solution containing 10 mgm. per ml. The oxidation and fractionation of insulin, and the partial hydrolyses of each fraction with both pepsins or hydrochloric acid were carried out under the conditions described by Sanger *et al.*[3]. The hydrolysates were fractionated by paper ionophoresis at *p*H 3·2 or 6·5 (pyridine–acetate buffer), and paper chromatography with phenol/ammonia and also *n*-butanol/acetic acid solvent mixtures. The peptides were eluted and the eluates hydrolysed with 6 *N* hydrochloric acid and the constituent amino-acids identified by paper chromatography. From a comparison between the amino-acid composition of these peptides and the known sequence of amino-acids in the *A* and *B* chains of beef insulin, it was possible to assign positions to