the delayed wound healing, decreased resistance to infection and accelerated vascular degeneration that occurs in diabetes mellitus.

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The intracellular distribution of enzymes in *Serratia marcescens*

The localisation of enzymes on particulate components of cytoplasm in the cells of higher animals and plants is well established. It has been shown in *Serratia marcescens* that the red water-insoluble pigment, prodigiosin, could be isolated as a protein complex[1]. This would imply some intracellular organization. It became of interest therefore to see if it could be shown that in bacterial cells there occurs also an organised distribution of enzymes on different cell fractions. This preliminary note describes the distribution of a number of enzymes between particulate and soluble fractions obtained from this organism.

The organisms were disintegrated by a high-speed mechanical shaker (5600 cycles/min)[2]. The capactity of the capsule in which the cells were disrupted is 20 ml; most effective breakage was obtained with 15 g of Ballotini glass beads size No. 14 and 1.5 g wet weight of *S. marcescens* suspended in 10 ml of 0.8 *M* sucrose, leaving an air space of about 3 ml. The capsule and contents were cooled to 0° prior to shaking in the cell disintegrator for 15–20 seconds, the temperature during the shaking rising about 5°. Approximately 10% of the cells were disrupted. Oscillation for longer periods lead to greater cell breakage but a different pattern of enzyme distribution. After diluting with 1.5 volumes of 0.3 *M* sucrose and removing the glass beads by centrifugation the supernatant was centrifuged for 20 minutes at 3,500 *g* and the residue discarded, and this procedure repeated twice so that the supernatant was cell-free. All centrifugations were carried out at 0°. The supernatant was then made 0.17 *M* with respect to KCl and centrifuged for 20 minutes at 25,000 *g*; the sediment and supernatant were collected separately, and subsequent reference to the "supernatant" refers to this fraction. The sediment was then washed once with the original volume of 0.5 *M* sucrose containing 0.17 *M* KCl and the washed sediment finally suspended in 0.5 *M* sucrose for enzymic assay. Viewed in a phase-contrast microscope the sediment was heterogeneous, being composed of particles and debris. The sediment is hereafter referred to as the particulate fraction. The particulate fraction was not composed of cell nuclei as shown by analysis of the desoxyribonucleic acid (DNA) distribution; the DNA was exclusively in the supernatant. Whether the particulate fraction represents discrete bodies within the cell, or mainly fragments of cytoplasmic membrane cannot be answered.

The distribution of a number of dehydrogenases between the supernatant and particulate fractions is shown in Table I. A disproportionate arrangement of the enzymes between the supernatant and particulate fractions is evident from Table I. It is possible to divide the enzymes associated with the particulate fraction into two groups: the first contains enzymes which are firmly attached to the particulate fraction, and the second enzymes easily dissociable from the particulate complex. The first group of enzymes is composed of succinic, α-ketoglutaric, lactic and formic dehydrogenases. The distribution of this group was not greatly affected by the disintegration period, being almost entirely concentrated in the particulate fraction under all conditions. Aconitase, fumarase, isocitric and malic dehydrogenases comprise the second group of enzymes, and with these the shaking period and the concentration of sucrose in which the cells were shaken was critical.

These enzymes were found on the particulate fraction only with a period of shaking less than 30 seconds and when a sucrose concentration of 0.8 *M* was maintained. If the sucrose concentration was 0.5 *M* or less, no aconitase or fumarase could be detected in the particulate fraction and diminishing amounts of malic and isocitric dehydrogenases were found. The shaking period was even more critical, prolonged shaking rendering all of the enzymes in this group soluble.

The supernatant fraction, although containing appreciable concentrations of aconitase, fumarase, isocitric and malic dehydrogenases could not oxidise aerobically any of these substrates. Just as in the case of animal cells the terminal oxidase system of *S. marcescens* is exclusively associated with the

TABLE I

DEHYDROGENASE ACTIVITIES IN SUPERNATANT AND PARTICULATE FRACTIONS OF *S. marcescens*

Usual THUNBERG technique, 38°. Each tube contained 0.003 *M* $MgCl_2$, 0.015 *M* K_2HPO_4/KH_2PO_4 buffer pH 7.3, 0.00003 *M* diphosphopyridine nucleotide and triphosphopyridine nucleotide (DPN–TPN), 0.008 *M* substrate, 0.5 *M* sucrose, enzyme in 0.5 *M* sucrose, 2 drops of liquid paraffin, 0.0003 *M* dichlorophenol indophenol and water to 3.0 ml. Activities are expressed as *Q* dye = μl dye decolorized/mg N/h.

Substrate	*Q* dye	
	Supernatant fraction	Particulate fraction
Nil	17	31
Citrate*	177	56
Isocitrate	510	81
α-Ketoglutarate	30	448
Succinate	41	830
Fumarate*	185	77
Malate	204	208
Lactate	24	167
α-Glycerophosphate	157	730
Formate	17	342

* The actual compounds dehydrogenated are malate and isocitrate.

TABLE II

OXIDATION OF SUBSTRATES BY SUPERNATANT AND PARTICULATE FRACTIONS

Manometry 30°. Each cup contained 0.003 *M* $MgCl_2$, 0.015 *M* K_2HPO_4/KH_2PO_4 buffer pH 7.3, 0.001 *M* adenylic acid, 0.00003 *M* DPN–TPN, 0.008 *M* substrate, 0.5 *M* sucrose, enzyme in 0.5 *M* sucrose, water to 3.3 ml. The values cited are corrected for the endogenous respiration.

Substrate	Q_{O_2} (N)	
	Particulate fraction	Supernatant and particulate fraction
α-Ketoglutarate	83	----
Succinate	175	—
Malate	115	88
Fumarate*	44	67
Citrate*	0	15

* In these experiments the manometers also contained a coenzyme concentrate prepared from yeast by the method of LE PAGE AND MUELLER[4] up to the first charcoal eluate.

Q_{O_2} (N) = μl O_2 uptake/mg N/h.

particulate fraction. Recombination of the supernatant and particulate fractions, however, did not lead to the oxygen uptakes predicted on the basis of enzyme concentration alone. Table II illustrates the salient points. The oxidation of citrate was unexpectedly low and could not be improved by any method adopted.

The low oxygen uptakes observed on recombination of the supernatant and particulate fractions may be explained in terms of dilution effects or in terms of enzyme orientation. Since the addition of coenzyme concentrates to the reaction mixture did not increase the rate of oxygen consumption, the latter explanation may be the more likely. It is considered from this work that aerobic oxidation within this bacterial cell may take place on particles distributed normally in the cytoplasm or that the enzymes are orientated on the cytoplasmic membrane. MOYED AND O'KANE[3] have shown the presence of a terminal oxidase system on particulate fractions isolated from *Proteus vulgaris*.

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