The molecular kinetic and electrophoretic behaviour of the protein appear to be independent of the method of preparation.

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July 11.

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Oxidation of Succinate by Heart Muscle

IT is widely held that the oxidation of succinate in animal tissues proceeds in the following manner:

$$\text{Oxygen} \longrightarrow \text{Cytochrome Oxidase} \longleftrightarrow \underset{(a,b,c)}{\text{Cytochromes}} \longleftrightarrow \left[\begin{array}{c}\text{Succinate} \rightleftarrows \text{Fumarate} \\ \text{Succinic dehydrogenase}\end{array}\right].$$

It is assumed that no co-enzyme or intermediary hydrogen carrier is required to link succinic dehydrogenase to the cytochrome-cytochrome oxidase system[1]. This question has acquired special significance in view of the essential role of the succinic-fumaric acid system in cellular respiration which has been postulated by Szent-Györgyi[2].

Oxidase preparations obtained by the method of Keilin and Hartree[3] from beef heart muscle extracts by isoelectric precipitation catalyse the reduction of methylene blue by succinate under anaerobic conditions as well as the oxidation of succinate by molecular oxygen. Upon repeated isoelectric precipitation, the aerobic activity towards succinate is greatly impaired, whereas that towards hydroquinone is unaltered; in both cases an excess of cytochrome *c* was added.

When phosphate extracts from ground and washed heart muscle are spun in an ultracentrifuge at 384 r.p.s. for 30 minutes, a sediment is obtained which does not catalyse the aerobic oxidation of succinate in spite of the fact that such preparations, even after twice repeated sedimentation, contain cytochrome oxidase, cytochrome *a*, *b* and *c*, and succinic dehydrogenase[4]. The ability to oxidize succinate aerobically is restored by the addition of small amounts of the clear, supernatant fluid obtained by the ultracentrifugation of crude heart muscle extracts. It exerts its activating power also towards oxidase preparations which have been partially inactivated by repeated isoelectric precipitation. The supernatant fluid alone is inactive towards succinate even in the presence of an excess of cytochrome *c*.

The activating principle present in the supernatant fluid is not dialysable, but it is inactivated upon dialysis against large volumes of phosphate buffer, *p*H 7·4. It is partly destroyed when the crude extract is kept at 100° for 10 minutes, and it is precipitated by trichloroacetic acid. The rate at which the principle sediments in the quantity ultracentrifuge at 512 r.p.s. (field at the bottom of the tubes, 69,000 *g*) suggests a molecular weight of the order of 140,000.

The activating principle is not identical with catalase[5] or with aluminium[6]. It may represent the factor the removal of which in the experiments by Stotz and Hastings[7] and in those of Hopkins, Lutwak-Mann and Morgan[8] led to partial or complete inactivation of their oxidase preparations towards succinate. On the other hand, spectroscopic observations show that succinate when added to ultracentrifugally purified oxidase preparations causes the reduction of the cytochromes *a*, *b* and *c* present in such preparations as well as that of added cytochrome *c*. The concept that *the activating principle provides a coupling link between succinic dehydrogenase and the cytochrome-cytochrome oxidase system* would be valid only if it can be shown that the rate of reduction of cytochrome by the incomplete enzyme system is insufficient to bring about a measurable oxygen uptake under the conditions of the manometric experiments. This question, as well as the possible relationship of the activating principle to a flavoprotein[9], is under investigation.

The results of typical experiments will be found in the accompanying table:

Preparation	Time for reduction of methylene blue at *p*H 7·4 and 37°		Oxygen uptake (*µl.* in 60 min.) in presence of 5 mgm. dry weight of enzyme preparation at *p*H 7·4 and 37°; 0·033 mM. hydroquinone and succinate respectively			
	Succinate	Control	Hydroquinone	Hydroquinone + Cytochrome	Succinate + Cytochrome	Succinate + Cytochrome + Activator
1 × ultracentrifuged	—	—	—	—	0	392
3 × ultracentrifuged	3 min.	>60 min.	60	700	18	960
1 × isoelect. precipitated	4 min.	>60 min.	—	720	545	—
3 × isoelect. precipitated	7 min.	>60 min.	—	760	120	—
3 × isoelect. precipitated, after 48 hr.	—	—	—	—	0	176

This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

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Poly-condensation of α-Amino Acid Esters

UNDER different experimental conditions, there can be obtained from glycine ethyl ester, among other known products, polypeptide esters of various chain lengths.

(1) Solutions of glycine ethyl ester in various solvents, kept either at room temperature or boiling point, deposit precipitates on long standing from which a number of fractions were isolated. The