A SIMPLE INEXPENSIVE BACTERIAL COLONY COUNTING DEVICE

by

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SIMS AND JORDAN[1] have described a method of counting bacterial colonies which utilises an electromagnetic device for automatic counting. Fig. 1 illustrates an apparatus simplified by using a mechanical counter in place of the electromagnetic type. The design of SIMS AND JORDAN is further improved upon by the built-in illumination provided.

The counter, *C*, is a Veeder model operated by a lever carrying two spring coils, S_1 and S_2, a Uno pen (Size 2) *U*, being attached to the coil by a piece of rubber tubing. The counting procedure is as follows:-

The pen, filled with suitable marking fluid, such as Indian ink, is pulled down towards the Petri dish to mark with a dot the position of a colony. Just before the pen reaches the dish the counter lever registers a count and simultaneously produces an audible click. The spring, S_2, enables the pen to reach any point on the dish, which remains in a fixed position. As the pen touches the dish, a second click is heard. Finally the spring S_1 returns the lever to its resting position (producing a third click) when the downward pull is released, ready for the next count. The rhythmic sequence of the three clicks on counting provides an audible proof of the faultless working of the procedure.

The Petri Dish, *P*, need not be marked out in squares, as there is no possibility of counting a colony twice or forgetting the number of colonies counted.

The lamp house, *H*, is made from a tin box, the bottom being removed and ventilation holes drilled near the top. A 25 Watt Lamp, *L*, gives the right lighting and very little heat. The Petri dish rests upon an opalescent plate, *O*, secured over a hole in the top of the box, and is thus viewed in diffuse light.

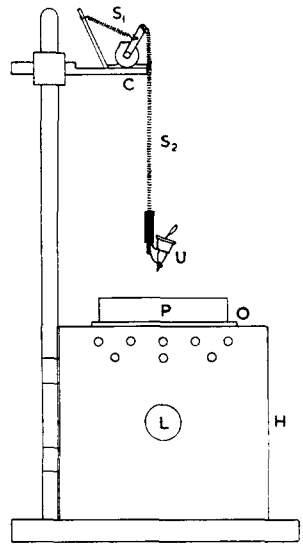

Fig. 1.

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ISOLATION OF SUCCINIC DEHYDROGENASE FROM BEEF HEART MITOCHONDRIA

by

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The main electron transport system of aerobic cells is associated with particles readily sedimentable in low gravitational fields and insoluble in aqueous media at neutral or acid pH[1]. Our present knowledge of this system is based almost exclusively on studies of the behaviour of mixtures

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of particulate enzymes containing all or most of the components necessary for transport of electrons from various substrates to molecular oxygen[1-3]. In recent years several investigators[4-8] have directed their efforts towards separating the different components of the electron transport system by way of the differential solubilization of particles with desoxycholic acid, and then fractionation of the mixtures of solubilized enzymes. The results obtained by these methods have clearly demonstrated the feasibility of separating the individual particulate components of the electron transfer system.

The present communication is the first in a series dealing with the problem of isolating particulate enzymes. The working hypothesis is that such enzymes after release from mitochondria are discrete entities in the same sense that a soluble enzyme is a defined chemical substance. The particulate enzymes differ from their soluble counterparts only in respect to the relatively high concentration of lyophobic groups attached to the protein molecule which conduce to complete insolubility in water.

The theory of the isolation procedures which we have developed is essentially the following. If two particles differ in size, shape or specific gravity they can be separated by differential centrifugation providing they do not associate. Given a chemical difference between two particles, a basis is provided for effecting differential changes in specific gravity since the extent to which particles will bind or associate with alcohols, salts and ions at different pH values will be determined by their respective chemical properties. These density differences then permit separation of the particles in the ultracentrifuge.

By application of a fractionation technique based on the principles discussed above succinic dehydrogenase has been isolated from beef heart mitochondria 15–20 times more active than the starting mitochondrial suspension. The following enzymes are absent from the preparation: cytochrome oxidase, DPNH dehydrogenase, cytochrome *c* and all the known enzymes of the citric acid cycle.

The dehydrogenase catalyzes the oxidation of succinate by ferricyanide, quinones, oxidation-reduction dyes and cytochrome *c* but not by molecular oxygen (even in presence of added cytochrome *c*). One mg of the lipin-free enzyme catalyzes the oxidation by ferricyanide of 7 μmoles of succinate per minute at 38°.

Assuming that antimycin A combines with one mole of succinic dehydrogenase[9] the value for the minimal molecular weight of the lipin-free enzyme is $8.3 \cdot 10^5$. Since the enzyme contains 61% by weight of lipin the uncorrected value for the molecular weight is *ca.* $2 \cdot 10^6$.

Each molecule of succinic dehydrogenase as defined above contains 4 molecules of hemin and 12–16 atoms of non-hemin iron. Neither copper nor flavin is present in detectable amounts. There is no evidence of the presence of cytochrome *c* in the enzyme as isolated. However each molecule of enzyme can bind up to 16 molecules of cytochrome *c*. Once bound, cytochrome *c* is not removable by repeated washing by centrifugation of the particulate enzyme.

In 1.5% desoxycholate solution the reduced enzyme shows 3 bands with maxima at 428, 523 and 558 mμ while the oxidized enzyme shows only one band with a peak at 415 mμ. The bands of the pyridine hemochromogen of the enzyme are indistinguishable from those of the pyridine hemochromogen of iron protoporphyrin — the maxima being at 420, 522 and 555 mμ.

On addition of succinate to the oxidized enzyme the prosthetic hemin is reduced at once. The reduced hemin is partially reoxidized by fumarate, completely reoxidized by quinone but not reoxidized by molecular oxygen. In presence of antimycin only half of the reducible prosthetic hemin is reduced at once by succinate—the other half being reduced only after some minutes. We have concluded from this observation that there are at least two types of hemin in succinic dehydrogenase —the reduction of one being antimycin insensitive and the reduction of the other antimycin sensitive.

It should be stated explicitly that no additions other than the particulate enzyme are necessary for the oxidation of succinate by cytochrome *c* and by any of the other electron acceptors. According to our evidence thus far the particulate succinic dehydrogenase which we have isolated is a complete unit within which the entire enzymic sequence proceeds.

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