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Short Communications

Cytochrome c in Sea-Urchin Eggs

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It is a matter of dispute whether the ordinary cytochrome system occurs in sea-urchin eggs. Runnström [1] found hemochromogen bands (512 and 548 mμ) in alcohol extracts, while a pyridine hemochromogen band at 550–560 mμ can be readily obtained [2, 3]. Although several workers [4–7] have unsuccessfully sought for cytochrome c, the bands of a and b have recently [8] been observed in eggs at liquid-air temperature. Studies of the effect of inhibitors on egg respiration [8–10] and experiments with cell extracts [7, 11, 12] have proved the presence of an oxidase closely allied to, or identical with, cytochrome oxidase. The ability of the extracts to oxidise cytochrome c strongly suggests the presence of cytochrome oxidase.

Cytochrome bands have been detected in sea-urchin spermatozoa [2, 3, 11] and Rothschild [13] has identified bands of the components a, a_3, b and c and also observed the CO-complex of a_3 (cytochrome oxidase). Recent studies of spectra at liquid-air temperature disclosed the presence of a component e [14].

In the present investigation suspensions of sea-urchin egg powder (*Echinus esculentus;* eggs extracted with acetone and ether, disintegrated in the Waring blendor, dried at room temperature) showed, after $Na_2S_2O_4$ addition, absorption bands at 605–600 (cytochrome a?) and 535–530 mμ, but no trace of cytochrome c bands. A very strong pyridine hemochromogen band (560 mμ) could be produced. It is not likely that the extraction and drying procedure has destroyed cytochrome c, since apparently the more sensitive cytochrome a is left unaffected, and, furthermore, acetone washing and drying have been used without ill-effects in ordinary cytochrome c preparation.

Krahl and co-workers [7] found that egg-extracts (*Arbacia*) had no specific absorption at 550 mμ, and could not replace cytochrome c in biochemical test systems. The authors concluded that the cytochrome c content, if any, was less than 10^{-2} μg per mg dry eggs. In the present investigation *Echinus* egg powder was analyzed for cytochrome c, using a new micromethod [15]. No cytochrome c was found, thus placing the upper limit of its concentration in the eggs at 5×10^{-4} μg per mg dry matter. Since the extractability of cytochrome c from dried egg material is not known this figure must be considered as approximate.

Baker's yeast has a Q_{0_2} round 200, and contains about 2.6 μg cytochrome c per mg dry matter [16]. Calculated from Borei's [17] figures (egg volume = 5.84×10^{-4} μl; dry matter round 20 %; oxygen consumption = 1.84×10^{-4} μl per egg and hour) the fertilized *Psammechinus* egg has a Q_{0_2} of 1.5. Accordingly, if the sea-urchin egg has a cytochrome system similar to that of baker's yeast, it would contain 2×10^{-2} μg cytochrome c per mg dry matter. Actually, however, the upper concentration limit probably lies much lower, as found above. This comparison is, however, only valid on assumption that there is a general proportionality between cytochrome c content and Q_{0_2} in different organisms.

Nevertheless, it must be born in mind that the oxidases of the echinoderm egg and of yeast or mammalian heart were found [12] to resemble each other very much. It is probable that the oxidase will have a different protein for each species (*cf.* hemoglobins), and that the proteins of the oxidases from heart and yeast may differ from each other just as much as either differs from that of echinoderm eggs. Furthermore, cytochrome c has been demonstrated in sea-urchin spermatozoa [13,14], and has been found to catalyze oxidations through the echinoderm egg oxidase [12]. A small amount of cytochrome c (apparently less than 5×10^{-4} μg per mg dry matter) is thus quite possible in the sea-urchin egg. In such a case the mammalian and the echinoderm cytochrome systems differ not in fundamental composition, but merely in concentration of their components.

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An Improved Method for Selfcondensations of Esters by means of Alkali Ethoxides

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A general method for the preparation of β-ketoesters of the formula $RCH_2COCH(R)CO_2C_5H_2$ is the forced self-condensation of esters with sodium ethoxide, as described by Mc Elvain [1–3]. The yields of the β-ketoesters obtained by this method are usually very good, but the practical procedure can be somewhat simplified.

The preparation of dry, powdered sodium or potassium ethoxide is a rather cumbersome process, but the preparation of a suspension of the same reagent is easily performed in an inert solvent whose boiling point is higher than the melting point of the metal, which can then be very finely divided and hence is very reactive.

The choice of the solvent is dependent upon the boiling point of the ester used. In order not to remove some of the ester by azetropic distillation with the alcohol formed in the reaction, the boiling point of the ester should be some degrees higher than that of the solvent. For esters with high boiling points, over 140°, xylene b. p. 135–140° is a useful solvent; for those in the region 115–140° toluene b. p. 111° can be used; and for those with a low boiling point, below 115°, the best solvent seems to be benzene, b. p. 81°, but in this case sodium m. p. 97.8° is preferrably replaced by potassium m. p. 63.5°. Of course sodium may be used if it is first powdered