DETECTION OF CENTERS OF SUCCINODEHYDROGENASE ACTIVITY IN Escherichia coli

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The survival of bacteria after freezing and drying is a problem of great importance in the preparation of live vaccines. The explanation of the causes of disturbance of the vital activity of the cell during intensive cooling and freeze-drying is of great theoretical and practical interest. The disturbance of vital activity during deep cooling may be connected with the denaturation of proteins, especially enzyme proteins, and with changes in the kinetics of metabolic processes during alterations in the temperature [5].

There are reports in the literature of the lability of certain enzymes connected with the mitochondrial fraction of animal tissues at a low temperature, among them certain dehydrogenases, notably succinodehydrogenase [1, 3, 6, 7, 8].

Since succinodehydrogenase is an important component of the final stage of cell respiration, any degree of denaturation of the protein of this enzyme may result in considerable changes in the vital activity of the cell. It is therefore important to investigate the changes in this enzyme in the bacterial cell when subjected to the action of low temperatures and dehydration.

EXPERIMENTAL METHOD

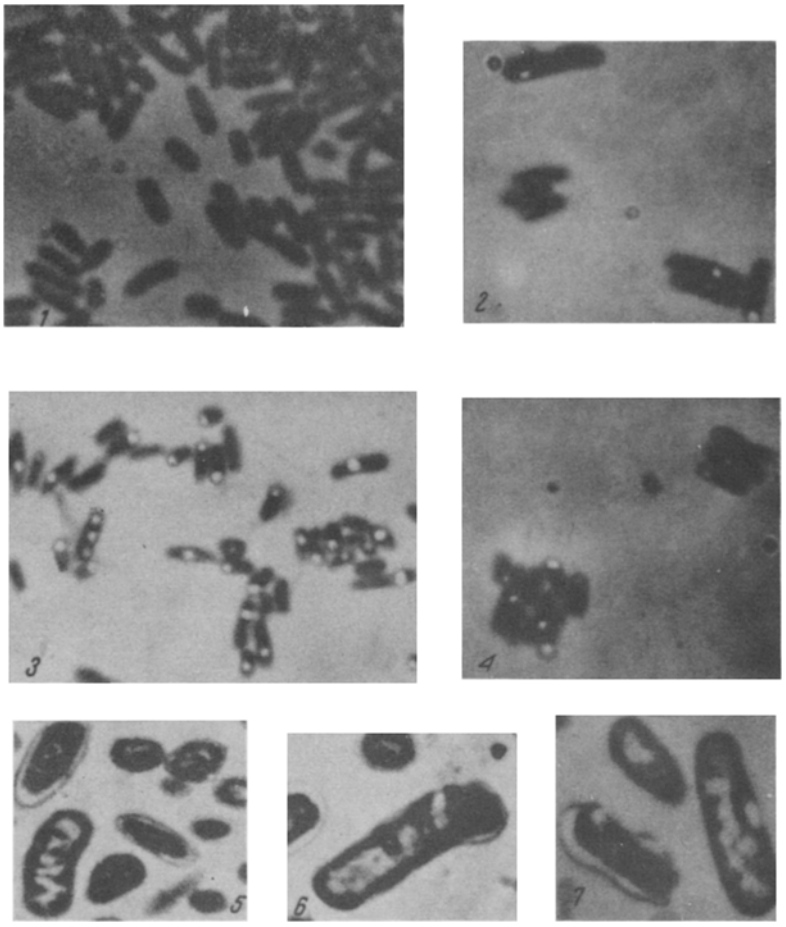
For the investigation, Escherichia coli strain 894 was used, which has typical morphological and cultural properties. Incubation was for 17.5 hr on meat-peptone agar at 37° with 2, 3, 5-triphenyltetrazolium chloride in the presence of sodium succinate in various concentrations and Ca^{++} ions (10^{-5} M) or cystine (M/2000-M/8000) as enzyme activators, or of sodium arsenite in various concentrations as inhibitor. Observations were made after different periods of incubation (from 10 to 60 minutes). Microscopy by means of a phase-contrast apparatus; objective FI90 ×, ocular 10 ×. Photography in the same conditions on a "Mikrat" film; exposure 8 seconds; photographic adaptor MFN-1 with MFK-3 camera. Focusing was carried out on formozan crystals, so that the cell contours were somewhat blurred. Part of the material was treated for preparation of ultrafine sections. Electron microscopy: microscope – ÉM-3, at 50 kv; magnification 5700-7300. The formozan was extracted by passage through alcohol, so that spaces where the crystals must have been are seen in the sections, and not the crystals themselves. The experimental material was investigated quantitatively with respect to formozan formation by the method of Fahmy and Walsh [2].

EXPERIMENTAL RESULTS

The specificity of the reaction was demonstrated. In the absence of sodium succinate in the washed E. coli suspension, no reduction of tetrazolium was observed after incubation for 60-120 minutes (see figure). In no case of work with a well-washed culture did we find formozan granules outside the cells.

After the addition of sodium succinate in different concentrations, the formation of formozan was observed (see figure, 2, 3). The higher the concentration of succinate, the more intensively was formozan formed. Ca^{++} ions and cystine activated the reaction. Arsénite depressed it significantly (see figure, 4). In the cells 1-2 formozan granules were most often found, distributed as previously described by Niklowitz [4], at both ends, or at one end and in the middle, close to the cell wall (see figure, 2). In one case we observed three formozan granules in the cell (see figure, 3). The grains were situated close to the cell wall (this is clearly seen in the early period of reduction, or after incubation with small concentrations of succinate).

In ultrathin sections of the bacteria the same localization is observed (by the wall; see figure, 5-7). The sites of reduction apparently border the cell membrane, being in the thickness of the cytoplasm. It cannot be concluded from our electron photomicrographs that the sites of reduction of tetrazolium differ in any way from



Reduction of triphenyltetrazolium chloride by Escherichia coli. 1) Control (E. coli 894 + tetrazolium chloride); 2) tetrazolium chloride + 0.005 M sodium succinate; 3) tetrazolium chloride + 0.05 M sodium succinate; 4) tetrazolium chloride + 0.05 M sodium succinate + $M/10^4$ sodium arsenite; 5) control (ultrathin sections); 6 and 7) incubation with 0.1% tetrazolium chloride.

the rest of the cytoplasm. Niklowitz [4] found that the respective areas could be detected morphologically in the cell before the reaction with tetrazolium.

The strict localization of the sites of succinodehydrogenase activity provides evidence of a definite morphological substrate of action of the enzyme. Since succinodehydrogenase activity is associated in tissues with the mitochondrial fraction, we may speak of such centers in the bacterial cell as functional equivalents of the mitochondria.

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SUMMARY

2, 3, 5-Triphenyltetrazolium chloride is a specific reagent for succinodehydrogenase in the Escherichia coli cell. A cell is found to have from 1 to 3 "activity centers" of succinodehydrogenase. Since the tissue succinodehydrogenase is associated with mitochondria, one may speak of mitochondria equivalents in the bacterial cell.

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