Nucleolar Localization of Succinic Dehydrogenase in Normal Rat Hepatic Cells

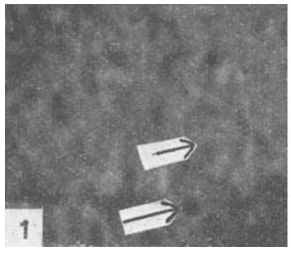
SCRUTINY of the literature[1–6] revealed that the oxidative enzyme, succinic dehydrogenase, is present in the mitochondria, occasionally in the cell membranes[2], of all mammalian cells. The absence of this enzyme in the nuclei including the nucleoli is a very characteristic feature, except in bird erythrocytes, which according to Brachet[7] may be due to the fact that bird erythrocytes have no mitochondria.

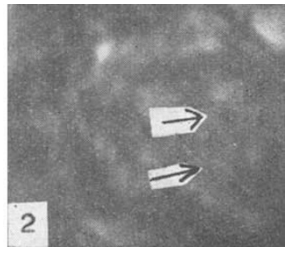
This report describes the cytochemical localization of succinic dehydrogenase in the nucleoli of normal rat hepatic cells besides their well-established cytoplasmic counterpart.

Frozen sections (8μ) of rat liver were cut, removing the endogenous substrates for succinic dehydrogenase in ice-cold 0·1 *M* phosphate buffer. Then the sections were incubated for 1 hr. at 37° C. in a medium containing 5 c.c. 0·1 *M* phosphate buffer *p*H 7·6, 5 c.c. 0·1 *M* sodium succinate, 5 c.c. distilled water and 10 mgm. triphenyl tetrazolium chloride. The common controls for confirming specificity of the reactions were applied[4,5,8]. The tetrazolium salt used in this experiment is considered to be very suitable for its high tissue penetrating power and the monochromatic nature of its formazan[9].

In the formazan-stained section, the same hepatic cell was studied with and without a phase-plate under a Zeiss phase-contrast microscope. During each of the two observations, photomicrographs were taken. Then the formazan colour was extracted from the above section by post-fixing it in Carnoy acetic alcohol and restained with pyronin methyl green for staining the nucleoli[10]. From the vernier readings of the mechanical stage recorded previously, pyronin methyl green-stained cells, in the same microscopic field as that of the formazan cells stained with formazan, were studied. A photomicrograph was taken.

It was observed that cytoplasmic formazan granules of rat-liver cells were stained in the usual manner; they showed the topography of mitochondria. The nuclei remained completely unstained and clear without any diffusion of cytoplasmic formazan inside them. In regard to shape, size and position, nucleolar-like formazan deposits of the same formazan-stained cell, with and without a phase-plate, yielded the same results as nucleoli extracted with formazan and stained by pyronin methyl green. The photomicrographs present evidence for nucleolar succinic dehydrogenase, and this unusual finding, besides the well-established cytoplasmic aspects of mitochondrial activity, is quite new.





Figs. 1 and 2. Arrows point to the nucleolar-like formazan deposits of the same formazan-stained hepatic cells without and with phase-contrast, respectively. (× 1,170)

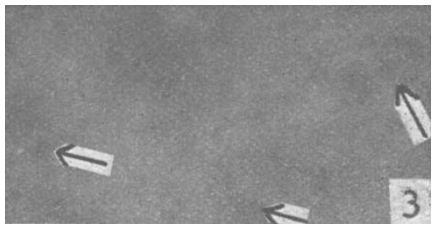


Fig. 3. Arrows point to the formazan-extracted and pyronin methyl green-stained nucleoli of hepatic cells of the same microscopic field (without phase-contrast). (× 1,340)

Thanks are due to Dr. S. K. Brahma, Dr. T. Mukherjee, Mr. S. Bose, Mr. S. Bhattacharya and Mr. N. Saha.

P. DE
R. CHATTERJEE
S. MITRA

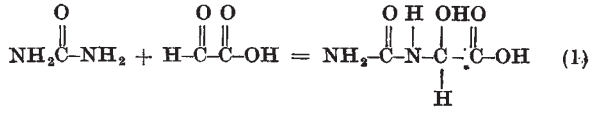
Cell Research Department,
Chittaranjan National Cancer Research Centre,
37 S.P. Mookerjee Road, Calcutta.

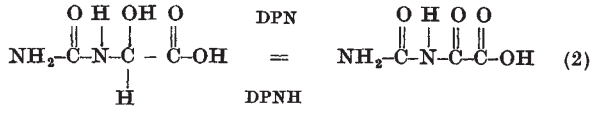
[1] Marcuse, P. M., *Lab. Invest.*, **6**, No. 2, 137 (1957).
[2] Monis, B., Nachlas, M. M., and Seligman, A. M., *Cancer Res.*, **12**, No. 6, 1238 (1959).
[3] Nachlas, M. M., Tsou, K. C., De Souza, E., Cheng, C. S., and Seligman, A. M., *J. Histochem. Cytochem.*, **5**, No. 4, 427 (1957).
[4] Ogawa, K., and Zimmerman, H. M., *J. Histochem. Cytochem.*, **7**, No. 5, 342 (1959).
[5] Pearson, B., and Defendi, V., *Cancer Res.*, **15**, No. 9, 593 (1955).
[6] Wachstein, M., and Meisel, E., *J. Biophys. Biochem. Cytol.*, **1**, No. 6, 484 (1955).
[7] Brachet, J., *Biochemical Cytology*, 94 (Academic Press, Inc., New York, 1957).
[8] Friede, R., *J. Histochem. Cytochem.*, **6**, No. 5, 350 (1958).
[9] Inversen, O. H., *Acta Path. et Microbiol. Scand.*, **47**, fasc. 3, 216 (1959).
[10] Gurr, R., *Methods of Analytical Histology and Histochemistry*, 157 (Leonard Hill (Books), Ltd., London, 1958).

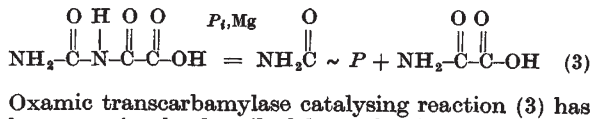
MICROBIOLOGY

Phosphate-dependent Degradation of Urea

Jones and Lipmann[1] recently proposed a direct mechanism for the phosphorolysis of urea. We have found that urea is phosphorolytically cleaved by extracts of *Streptococcus allantoicus*. However, the urea moiety is indirectly decomposed as made evident by the requirement for glyoxylate. Co-factors for the following scheme of urea degradation in which glyoxylurea is an intermediate are diphosphopyridine nucleotide (DPN), magnesium, and phosphate or arsenate :


(1)


(2)


(3)

Oxamic transcarbamylase catalysing reaction (3) has been previously described by Valentine and Wolfe[2].

Procedures used for growth of *S. allantoicus* and preparation of cell-free extracts have been described previously[2,3]. Degradation of urea was measured under the following conditions : a suitable amount of an enzyme fraction[2] was incubated with 100 μmoles