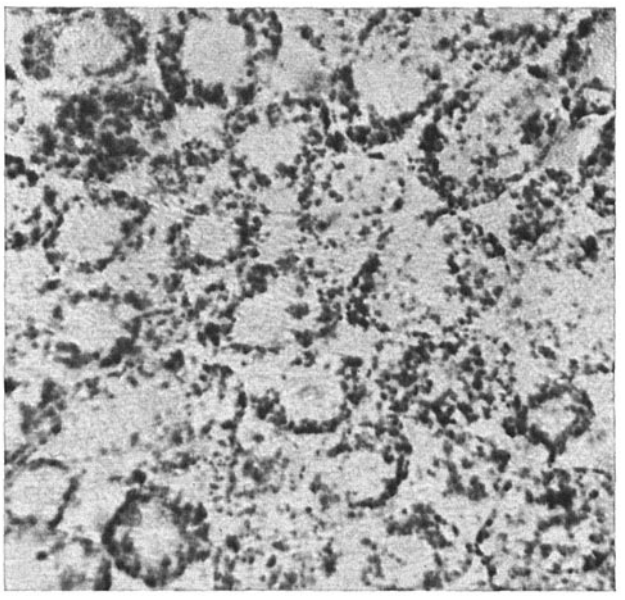
PRO EXPERIMENTIS

Histochemical Demonstration of Succinic Dehydrogenase in Cryostat Sections of Fresh-Frozen Plant Tissues

Succinic dehydrogenase (SDH), an important enzyme of the citric acid cycle, catalyzes the dehydrogenation of succinate to fumarate. Intracellularly the enzyme is localized in the mitochondria; this was shown on plants both biochemically, by the isolation of the enzyme from mitochondria[1], and electron microscopically[2].

ROBERTS et al.[3] investigated the SDH histochemically in cryostat sections of plant tissues. Freezing inactivated the enzyme. The activity could be restored by phenazine methosulphate (PMS) if cryostat sections were prepared only after the histochemical demonstration of SDH in unsectioned tissue blocks. We tried to reproduce the results of ROBERTS and compared them with our earlier investigations[4].

Pieces of unfixed root meristems were embedded in fresh animal tissue as a supporting medium, frozen with CO_2, and sectioned in a cryostat at -17 °C (14–16 μ)[4]. Similarly to ROBERTS, we used a modified tetrazolium salt method according to NACHLAS et al.[5,6] to show SDH activity. Sections were incubated in the substrate at 37 °C for 1 h with the tetrazolium salt tetra-nitro-BT (TNBT)[7].



Intracellular localization of succinic dehydrogenase in the mitochondria without addition of phenazine methosulphate to the incubation medium (cryostat section through the root meristem of *Zea Mays*). ×650.

Without PMS in the incubation medium, the SDH is localized in the mitochondria (Figure). The intensity of staining is greatly enhanced by PMS. Moreover, a diffuse staining appears in cytoplasm. HASHIMOTO et al.[8] achieved similar results with animal material. These authors have suggested that the mitochondria are damaged during histochemical procedure, then a hydrogen-carrier is released and reduces PMS, resulting in unspecific formation of formazan in the cytoplasm. Therefore, one has to add PMS only by weak enzyme activities. In this case, a short incubation time must be allowed to avoid unspecific staining in the cytoplasm. Furthermore, it must be mentioned that an exact localization of SDH is only possible with the tetrazolium salt TNBT.

ROBERTS incubated his sections at 20 °C. Warmth gives the activation energy for the enzyme reaction. The optimal temperature for SDH activity is about 37 °C; therefore ROBERTS was able to demonstrate weak activities of SDH at 20 °C only by adding PMS to the substrate.

It follows that the SDH is not inactivated by freezing, its activity is only diminished. PMS works as a mediator or hydrogen-carrier for the transfer of hydrogen to the tetrazolium. Reduced PMS reduces TNBT directly and not enzymatically.

The cryostat technique for fresh plant tissues[4] guarantees an exact localization of SDH in the mitochondria and is very suitable for the demonstration of other dehydrogenases and the aliesterase[4], and also for the acid phosphatase[9].

Zusammenfassung. Die Bernsteinsäuredehydrogenase in Pflanzen ist histochemisch in den Mitochondrien nachweisbar. Die Darstellung sehr schwacher Enzymaktivitäten gelingt unter Zusatz von Phenazinmethosulfat. Dieses wirkt als Wasserstoffträger für die H_2-Übertragung auf das Tetrazolsalz.

A. LÄUCHLI

Botanical Institute, University of Basel (Switzerland), 2nd November 1966.

[1] A. J. HIATT, Pl. Physiol., Lancaster *35*, Suppl. xi (1960).
[2] C. J. AVERS and M. M. TKAL, J. Histochem. Cytochem. *11*, 157 (1963).
[3] L. W. ROBERTS, S. BABA and K. URBAN, Pl. Cell Physiol., Tokyo *7*, 177 (1966).
[4] A. LÄUCHLI, Planta *70*, 13 (1966).
[5] M. M. NACHLAS, K. C. TSOU, E. DE SOUZA, C. S. CHENG and A. M. SELIGMAN, J. Histochem. Cytochem. *5*, 420 (1957).
[6] A. G. E. PEARSE, *Histochemistry, Theoretical and Applied*, 2nd Edn (J. and A. Churchill, London 1960).
[7] W. MEIER-RUGE, Histochemie *4*, 438 (1965).
[8] T. HASHIMOTO, J. S. KALUZA and M. S. BURSTONE, J. Histochem. Cytochem. *12*, 797 (1964).
[9] A. LÄUCHLI, Naturwissenschaften *53*, 533 (1966).

Die Probeentnahme in kinetischen Stoffwechseluntersuchungen mit Wasserlinsen *Lemna minor* L. (Lemnaceen)

Auf die Vorzüge der Lemnaceen als Laborpflanzen wurde bereits mehrfach hingewiesen[1–4]. Über ihre Verwendung in Gaswechsel- und Photosyntheseuntersuchungen werden wir an anderer Stelle berichten. Als besonders geeignetes Versuchsmaterial für kinetische Stoffwechseluntersuchungen nehmen diese kleinsten der Angiospermen wegen der raschen vegetativen Vermehrung und der leicht erzielbaren genetischen und physiologischen Homo-