lation between endogenous NADH_2 and cytochrome *b*. Thus it appears that electron transport from exogenous NADH_2 is again different from endogenous NADH_2 and is not coupled to the first site of oxidative phosphorylation. It seems reasonable to suggest that the rotenone-insensitive oxidation of exogenous NADH_2 found in Jerusalem-artichoke mitochondria may also not be coupled to oxidative phosphorylation at the first coupling site, resulting in the lower P/O ratio. These results could be explained on the basis of the chemiosmotic theory if the dehydrogenase responsible for the oxidation of exogenous NADH_2 is located on the outside of the coupling membrane, where it would fail to translocate protons during the first loop of electron transport (Mitchell, 1967). The results of initial experiments with ferriyanide as a non-penetrating electron acceptor are consistent with the localization of the dehydrogenase on the outside of the coupling membrane.

This work was supported by a research grant from the Scientific Research Council.

- Bonner, W. D. & Bendall, D. S. (1968). *Biochem. J.* **109**, 47p.
 Hackett, D. P. & Haas, D. W. (1958). *Pl. Physiol., Lancaster*, **33**, 27.
 Ikuma, H. & Bonner, W. D. (1967). *Pl. Physiol., Lancaster*, **42**, 67.
 Mitchell, P. (1967). *Fedn Proc. Fedn Am. Soc. exp. Biol.* **26**, 1370.
 Srivastava, H. K. & Sarkissian, I. V. (1970). *Physiologia Pl.* **23**, 63.

Polyamine Oxidase in Higher Plants

By T. A. SMITH and R. A. J. STEVENS. (*Long Ashton Research Station, Bristol BS18 9AF, U.K.*)

Although the polyamines spermidine and spermine are widespread in higher plants (Smith, 1970a), specific enzymes oxidizing these substances in plant tissue have not been reported.

By using gas chromatography, peaks indistinguishable from 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline were found in extracts of both seedling and mature leaves of barley (Smith, 1970b), suggesting the presence of a spermine oxidase (spermine- O_2 oxidoreductase, EC 1.5.3.3), similar to an enzyme demonstrated in the bacterium *Neisseria perflava* (Weaver & Herbst, 1958).

Reputedly authentic 1-(3-aminopropyl)pyrroline has been prepared by the catalytic oxidation of spermine (Wrede, 1926; Wrede, Fanselow & Strack, 1926) and by the enzymic oxidation of spermidine (Hasse & Schührer, 1962). Presumed 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline formed on oxidation of spermine by the barley and maize enzymes were indistinguishable from the

synthetic amines by thin-layer and gas-liquid chromatography.

Leaves of barley and maize seedlings were extracted into water (2vol.), acidified to pH 4.0 with HCl, centrifuged (1000g, 15min) and readjusted to pH 6.0 with NaOH. Ammonium sulphate was then added to 50% saturation and the precipitate was dialysed against buffer. Activity was determined by oxygen uptake in the presence of catalase, added to destroy the hydrogen peroxide formed, and also by gas chromatography of the products, 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline. For barley, the optimum pH was 4.5 with spermine as substrate, but with spermidine and 3,3'-diaminodipropylamine the optimum was about pH 7.0. The activity with the latter substrates at pH 7.0 was less than 5% of that with spermine at pH 4.5. Stoichiometry of the reaction is difficult to establish, since oxygen uptake exceeds the expected value.

Spermidine and spermine (10mm) were fed separately to intact 7-day-old barley leaves for 24h and the amine fraction (Smith, 1970a) was subjected to gas chromatography. Spermidine gave an increase in 1,3-diaminopropane, putrescine and 1-(3-aminopropyl)pyrroline, and spermine gave an increase in 1,3-diaminopropane and 1-(3-aminopropyl)pyrroline.

Enzyme activity in maize seedlings was localized histochemically. Tissue sections were immersed in a reagent containing soluble starch (1.3%), KI (20mm) and spermine tetrahydrochloride (8mm). Development of a blue colour due to enzymically generated hydrogen peroxide indicated spermine oxidase activity. Most activity was found in the vascular tissue (excluding the xylem) in the leaves, roots and especially the mesocotyl. No colour developed in controls omitting any one component, nor would putrescine substitute for spermine.

R. A. J. S. thanks the Imperial Tobacco Company for financial support.

- Hasse, K. & Schührer, K. (1962). *Biochem. Z.* **336**, 20.
 Smith, T. A. (1970a). *Phytochemistry*, **9**, 1479.
 Smith, T. A. (1970b). *Ann. N.Y. Acad. Sci.* **171**, 988.
 Weaver, R. H. & Herbst, E. J. (1958). *J. biol. Chem.* **231**, 647.
 Wrede, F. (1926). *Hoppe-Seyler's Z. physiol. Chem.* **153**, 291.
 Wrede, F., Fanselow, H. & Strack, E. (1926). *Hoppe-Seyler's Z. physiol. Chem.* **161**, 66.