Polyphenolases in the 1000 *g* fraction of *Papaver somniferum* latex

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Work by Fairbairn, Palmer & Patterson (1968) and Fairbairn & Djote (1970) has shown that morphine can by synthesized by the latex from tyrosine and dopa and that this is associated with the latex fraction which sediments at 1000 g. Reports of the occurrence of polyphenolase in latex (Meissner, 1966a,b) together with the fact that various workers (Barton & Cohen, 1957) have considered the enzyme complex as possibly responsible for the oxidative coupling reactions involved in the biosynthesis of the alkaloids, have resulted in the present work on polyphenolase in poppy latex and its association with alkaloid biogenesis.

Poppy latex was separated into a 1000 *g* fraction (A), an 11000 *g* fraction (B) and the supernatant (C). The whole of the detectable polyphenolase activity resided in the 1000 *g* fraction. Treatments used designed to rupture the membranes of the organelles were (1) lowering of the osmotic pressure of the solution of organelles below 0·3M (2) freeze/thawing (3) sonication and (4) solubilization with 0·1% Triton X-100. The oxidation of the phenolic substrate (catechol) increased with increased fragmentation of the organelles, the greatest activity being observed with the use of Triton X-100. These experiments also showed that both enzyme and substrate occur within the organelle and therefore indicate compartmentalization within the organelle. The activity of the polyphenolase was inhibited with KCN and DIECA at concentrations of 10^{-4}M. Experiments indicated that up to 50% of the enzyme activity was strongly membrane bound. The substrates oxidized by latex polyphenolase at pH 8·0 were caffeic acid, catechol, *p*-coumaric acid, *p*-cresol, dopa, hydroquinone, hydroxytyramine and tyrosine. No oxidation was observed with ferulic acid, guaiacol, *p*-hydroxybenzoic acid, 2,6,methoxyphenol, ± reticuline, salutaridinol and vanillic acid. These results show that the latex 1000 *g* organelles contain catechol oxidase (Ec. 1.10.3.1) and also give evidence of both tyrosinase and laccase activities. Since this enzyme will not oxidize phenols containing a methoxy-group in the *ortho*-position, it is perhaps not surprising that the intermediates of morphine biosynthesis, (±)-reticuline and salutaridinol were not oxidized. The present evidence also indicates that the 1000 *g* organelles of poppy latex are not lysosomes (de Duve, 1959, Pujarniscle, 1968) nor are they similar to the peroxisomes of Tolbert, Oeses & others (1968) or the glyoxosomes of Cooper & Beevers (1969).

REFERENCES

BARTON, D. H. R. & COHEN, T. (1957). Festschrift A. Stoll, Basel. 117.
COOPER, T. G. & BEEVERS, H. (1969). *J. biol. Chem.*, **244**, 3507.
DE DUVE (1959). *Subcellular Particles.* p. 128–157. Ronald Press Co., N.Y.
FAIRBAIRN, J. W. & DJOTE, J. (1970). *Phytochem.*, **9**, 739.
FAIRBAIRN, J. W., PALMER, J. M. & PATERSON, A. (1968). *Phytochem.*, **7**, 2117.
MEISSNER, L. (1966a). *Flora Abt. A. Bd.*, **157**, 1–26.
MEISSNER, L. (1966b). *Ibid.*, **156**, 634–654.
PUJARNISCLE, A. (1968). *Physiol. Veg.*, **6**, 27–46.
TOLBERT, N. E., OESES, A., KISARKI, T., HAGEMAN, R. H. & YAMAZAKI, R. K. (1968). *J. biol. Chem.*, **243**, 519.

The preservation of ophthalmic solutions with antibacterial combinations

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Preserved solutions of pilocarpine hydrochloride (1·0%) and of atropine sulphate (1·0%) were sterilized by autoclaving at 115° for 30 min while preserved solutions of physostigmine sulphate and of salicylate (0·25%) were sterilized by heating at 98–100° for 30 min. Preservatives used were benzalkonium (0·01%), chlorhexidine (0·01%), phenylmercuric nitrate (PMN) (0·002%), chlorocresol (0·05%) and chlorbutol (0·5%) as simple solutions, and also as combinations with either phenylethanol (0·4%) or disodium edetate (EDTA) (0·05%). Solutions were contaminated on two separate occasions with 10^6–10^7 cells/ml from overnight cultures of *Pseudomonas aeruginosa* NCTC 6750.