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.

Phenylethanol-antibacterial combinations killed the inoculum within 15 min except for one formulation with chlorhexidine and physostigmine salicylate (30 min), and all formulations with PMN (45–90 min). Nevertheless simple solutions of antibacterial alone in these formulations had much slower sterilization times than the phenylethanol combinations.

Benzalkonium with pilocarpine and with physostigmine sulphate had sterilization times within 15 min, but with atropine the time was 60 min. Benzalkonium is either less effective in atropine solutions than in solutions of the two other alkaloids or the inoculum into the atropine had higher intrinsic resistance. The phenylethanol-benzalkonium and EDTA-benzalkonium combinations, however, were both effective within 15 min.

Chlorbutol in simple solution has slower sterilization times with physostigmine salts (30 and 45 min) than with pilocarpine and atropine (15 min). This can be explained in terms of pH. The pH values of the autoclaved solutions are in the range 2·2–2·4 but the range for the steamed solutions is 3·1–3·6.

EDTA-PMN, EDTA-chlorbutol and EDTA-chlorocresol combinations show no clear advantage over the antibacterials in simple solution. The mode of action of the antibacterial agent and the state of resistance of the *P. aeruginosa* may determine whether EDTA enhances antibacterial activity or not.

Chlorhexidine had a slow sterilization time of 180 min with physostigmine salicylate. The sodium metabisulphite in the preparation may be reducing the effectiveness of the chlorhexidine. Phenylethanol-chlorocresol and phenylethanol-chlorbutol combinations sterilize physostigmine salicylate within 15 min.

These results, in conjunction with previous work (Richards, Suwanprakorn & others, 1969; Richards & McBride, 1971a,b; Richards, 1971) support the use of phenylethanol 0·4% in combination with other antibacterial agents in the preservation of ophthalmic solutions against contamination with *P. aeruginosa.*

REFERENCES

RICHARDS, R. M. E. (1971). *J. Pharm. Pharmac.* In the press.
RICHARDS, R. M. E. & MCBRIDE, R. J. (1971a). *Brit. J. Ophthal.* In the press.
RICHARDS, R. M. E. & MCBRIDE, R. J. (1971b). *J. Pharm. Pharmac.* In the press.
RICHARDS, R. M. E., SUWANPRAKORN, P., NEAWBANIJ, S., & SURASDIKUL, N. (1969), *Ibid.*, **21**, 681–686.

Effects of drying on polymyxin sensitivity of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa cultures dried over P_2O_5 during studies on cell wall composition were about 200-fold more resistant to polymyxin than before desiccation. Webb (1967) has reported possible mutagenic effects of desiccation on *Escherichia coli.*

P. aeruginosa strains NCTC 6750 and 1999, NCIB 8625 and several laboratory strains were cultured at 37° in nutrient broth either 100 ml in flasks in a shaking water bath or in 8 litre stirred magnetically. Cells were harvested by centrifugation, unwashed or washed three times in 0·9% NaCl, and the pellets stored in a vacuum desiccator over P_2O_5. The minimum inhibitory concentration (MIC) of polymyxin B sulphate (units/ml) in broth using inocula of 10^6 (total count) in final volume of 5 ml was measured before and after drying. The MIC increased from about 10–20 units/ml to over 2000 units/ml. These increases in resistance occurred with all strains on several occasions. With *P. aeruginosa* NCTC 6750 several consecutive attempts to increase resistance by vacuum drying were unsuccessful although previous and subsequent attempts using the same procedures were successful with this strain.

The resistance persisted through repeated subculture and was associated with colonial variants. These were small cream-yellow colonies similar in appearance to polymyxin resistant mutants obtained by selection. Colonies of both kinds occurred after drying and were picked off the surface of agar plates, diluted and standardized by optical density and the MIC measured. Typical green colonies were polymyxin sensitive and cream-yellow colonies were resistant. Colony plate counts and most probably number estimations in broth showed