PRELIMINARY NOTES

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The intracellular localization of exonuclear and endonuclear "phosphodiesterase" activity by histochemical methods

The present state of knowledge concerning the intracellular localization of the polynucleotidases, *i.e.*, those enzymes that attack oligo- and polynucleotides, is due largely to cellular fractionation techniques The difficulties encountered in obtaining comparable evidence by standard histo- and cytochemical means which, incidentally, apply as well to cellular fractionation techniques, were outlined by SIERAKOWSKA AND SHUGAR[1,2] Moreover, a procedure for the histochemical demonstration of kidney orthophosphoric diester phosphohydrolase (EC 3 1 4.1, phosphodiesterase I) was reported by these authors[3] who utilized a specific synthetic substrate, naphthyl-pT The method is based on a standard azo dye coupling of enzymically liberated α-naphthol On the other hand, attempts to localize spleen phosphodiesterase II with Tp-naphthyl, proved to be unsuccessful The resistance of the substrate to enzymic attack stands in contrast to the reasonably facile hydrolysis of Tp-nitrophenyl by phosphodiesterase II[4,5] This difference has been ascribed to an increased steric requirement of the α-naphthyl moiety[2].

Recent studies in this laboratory have led to the histochemical demonstration of both acid and alkaline phosphomonoesterase[6] (orthophosphoric monoester phosphohydrolase, EC 3 1 3 2, and EC 3.1 3 1, respectively) through the use of the synthetic substrate *p*-toluidinium 5-bromo-4-chloro-3-indolyl phosphate[7] Enzymic hydrolysis of this substrate liberates 5-bromo-4-chloroindol-3-ol, which is rapidly and irreversibly oxidized to 5,5′-dibromo-4,4′-dichloroindigo at the sites of activity

On the basis of these findings, efforts were directed to the application of the indigogenic principle to the histochemical localization of phosphodiesterases I and II The syntheses, which were modeled after those described by RAZZELL AND KHORANA[8] for nitrophenyl-pT and Tp-nitrophenyl, proceeded from 1-acetyl-5-bromo-4-chloroindol-3-ol (ref 9) to give 5-bromo-4-chloro-3-indolyl-pT and Tp-5-bromo-4-chloro-3-indolyl in moderate yields.

Phosphodiesterase I activity was observed at pH 9 2 (Tris buffer) with 5-bromo-4-chloro-3-indolyl-pT which affords a granular cytoplasmic deposition of the indigo in the proximal and distal convoluted tubules of rat kidney, liver, gastrointestinal tract and epididymus (Fig 1) Cytoplasmic activity was heavy in the brush border zone of kidney tubules with no evidence of nuclear activity. These observations are in essential agreement with earlier histochemical findings[3].

By contrast, Tp-5-bromo-4-chloro-3-indolyl produced a marked histochemical reaction at pH 4.8–5 2 (acetate buffer) in tissues of rat and mouse that was localized chiefly in cell nuclei and was particularly prominent in the spleen (Fig. 2A). At pH 5 9 (acetate buffer), nuclear staining was markedly reduced, instead the reaction was observed mainly in cytoplasmic granules of reticulum cells in liver, spleen, gastro-

Abbreviations reference is made in the text to several aryl-pT and Tp-aryl derivatives where pT and Tp are thymidine 5′-phosphate and thymidine 3′-phosphate, respectively

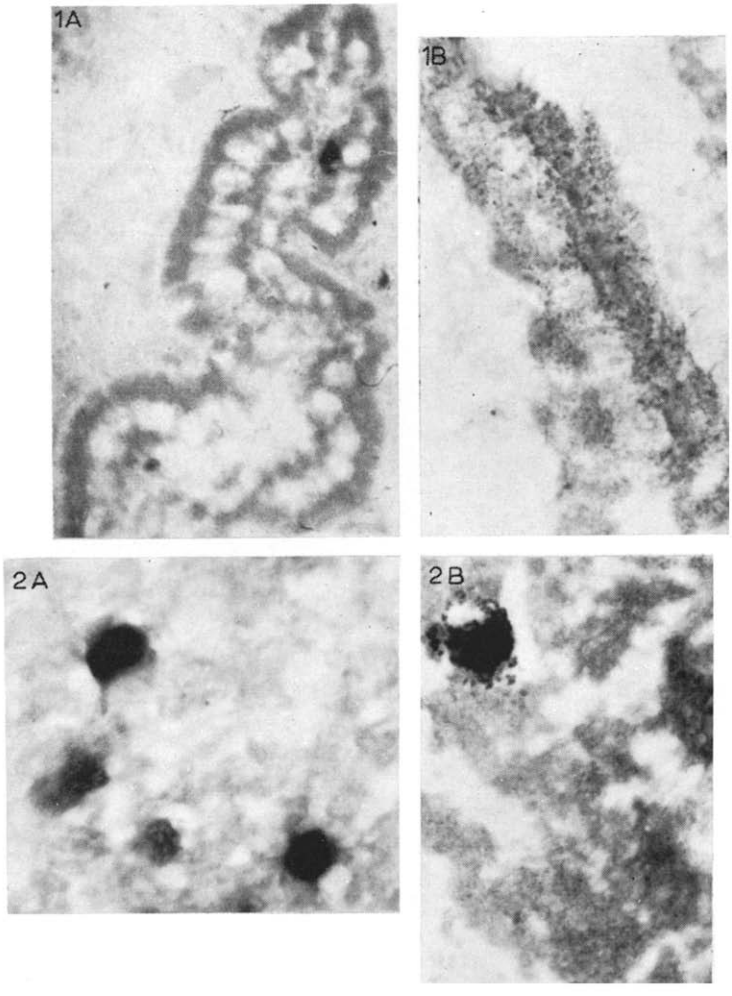

Fig 1 A. An unfixed frozen section of rat small intestine demonstrating granular cytoplasmic deposition of indigo in the cytoplasm of the mucosal cells and the reticulum cells The indigo is at the site of phosphodiesterase I activity at pH 9 0 utilizing 5-bromo-4-chloro-3-indolyl-pT × 400 B Higher magnification (× 1200) of A emphasizing the granular nature of indigogenic staining

Fig 2 A An unfixed frozen section of rat spleen demonstrating marked deposition of indigo in nuclei of reticulum cells at periphery of follicle There is minimal cytoplasmic staining The substrate utilized was Tp-5-bromo-4-chloro-3-indolyl at pH 5 2 × 1200 B An unfixed frozen section of rat small intestine demonstrating marked cytoplasmic and granular droplet deposition of indigo in mucosal cells and reticulum cells at pH 5 9 The nuclei are negative The substrate utilized was Tp-5-bromo-4-chloro-3-indolyl × 1200

intestinal tract and lung (Fig 2B). The addition of the redox system potassium ferro- and ferricyanides, which is frequently included in the incubation medium to effect the oxidation of the intermediate indoxyl to indigo[10], leads to a significant inhibition of staining of the cytoplasm

Recently, BERNARDI and co-workers[11,12] have shown that a highly purified form

of hog spleen acid deoxyribonuclease (deoxyribonucleate 3'-oligonucleotidohydrolase, EC 3 1 4.6, deoxyribonuclease II) shows "phosphodiesterase" activity toward a series of *p*-nitrophenylphosphodiesters These findings, together with the histochemical demonstration of both exo- and endopolynucleotidase(s) in the spleen as well as other tissues of the rat and mouse with Tp-5-bromo-4-chloro-3-indolyl, cast doubt on the currently accepted procedure for the specific assay of phosphodiesterase II with Tp-nitrophenyl[13]

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