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SHORT COMMUNICATIONS

Histochemical demonstration of histamine in paraffin sections by a fluorescence method

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LAST year Ehinger, Håkanson, Owman and Thunberg[1,2] developed a fluorescence microscopic technique with a sensitivity high enough to demonstrate, for the first time, also non-mast cell histamine. The procedure is based on the *o*-phthaldialdehyde method of Shore *et al.*[3] used for the quantitative fluorometric determination of histamine. This test-tube method could be introduced into histochemistry by applying the nearly dry reaction conditions originally described by Falck and Torp[4] for the cellular localization of catecholamines. Thus, fresh cryostat sections were freeze-dried, exposed to *o*-phthaldialdehyde vapour, followed by slight humidification.[1,2] Besides in mast cells, an intense specific fluorescence was found to develop in numerous epithelial cells located basally in the glandular part of the murine stomach.[2,5,6] Morphologically they resembled the serotonin-containing enterochromaffin cells present in the pylorus region, but they differed in that they contained no histochemically demonstrable monoamines.[2] The failure of other investigators[7] to visualize non-mast cell histamine in the stomach by similar histochemical applications of the *o*-phthaldialdehyde method is probably due to extraction and diffusion of the amine and/or the fluorophore in the tissues as a result of less strictly anhydrous conditions.

The specificity of the *o*-phthaldialdehyde method has been investigated in model systems,[1,8] by microspectrographic analysis of the fluorophores in tissues[9] and by parallel determinations of histamine and histamine-forming capacity.[5,6]

It has now been made possible to apply the method to paraffin-embedded tissues, thus simplifying the procedure compared with cryostat sectioning: The tissue pieces are dissected out and rapidly frozen to the temperature of liquid nitrogen in a propane–propylene mixture, followed by freeze-drying according to any conventional technique.[10] After embedding in fresh paraffin *in vacuo* (10 min at 60°) microtome sections are prepared and placed on a slightly pre-heated slide, allowing the sections to stretch in their own melting paraffin. After cooling, the sections are de-paraffinized by applying a few drops of xylene and then dried with a filter paper. A closed glass jar (approximately 500 cc), containing a few mg of *o*-phthaldialdehyde freshly recrystalized from heptane, is pre-heated in an oven at 100° for 10 min to allow vapourization. The slides with the sections are placed in the jar which has been removed to room temperature; it is again closed and the sections are treated for 90 sec. They are taken out and exposed to the steam from boiling water for 5 sec and then placed for 5 min in an oven at 80° for drying and stabilization of the fluorophores. The sections are mounted in xylene and analyzed in a fluorescence microscope. The fluorophores are activated with the 365 mμ mercury line from a HBO 200W high pressure lamp, and emit a blue or—probably in combination with more humid reaction conditions—a yellow light of high intensity.

The *o*-phthaldialdehyde method for paraffin sections appears to have the same degree of specificity and sensitivity as the original cryostat technique. However, the sections can be made thinner, they are better preserved than the cryostat sections and the fluorophores are therefore less easily diffused from their original cellular site upon humidification, which is an essential step in the present procedure. This gives the paraffin method a higher degree of precision and reproducibility.

The procedure has been used for the demonstration of histamine in mast cells (Fig. 1) from a variety of normal tissues from several species including man, as well as in human keloids and canine mastocytomas. Histamine can also be demonstrated in other cell systems, such as the previously described "enterochromaffin-like" cells in the acid-secreting region of the murine stomach (Fig. 2), or in hitherto unidentified cell populations in the anterior pituitary and islets of Langerhans. However, it should be emphasized that, in order to secure specificity of the reaction, it is necessary to combine

the histochemical procedure with parallel quantitative determinations of histamine in the tissues until the chemical reactions underlying the method are fully understood.

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Influence du β-mercaptoethanol sur certains enzymes plasmatiques du rat

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L'INJECTION de substances radioprotectrices à fonction SH ou SS provoque-rapidement chez le rat, des troubles métaboliques importants ainsi que des lésions des mitochondries et du réticulum endoplasmique des cellules de la muqueuse intestinale, et de la pulpe rouge de la rate.[1,2,4] Ces lésions bien visibles au microscope électronique, sont réversibles 90 à 120 minutes après l'injection.

Parallèlement, la teneur du plasma en divers enzymes intracellulaires: déshydrogénase lactique (LDH, localisation cytoplasmique), déshydrogénase glutamique (GlDH, localisation exclusivement mitochondriale), déshydrogénase malique et transaminase glutamique (MDH et TGO, toutes deux à localisation cytoplasmique et mitochondriale) et β-glucuronidase (enzyme lysosomial) augmente, l'élévation maximale est atteinte à 120 minutes, le retour à la normale s'effectuant en 6 H.[3]

Il semble donc que l'injection d'un radioprotecteur soufré déclenche un choc cellulaire intense mais rapidement réversible.

Comme Hugon, Maisin et Borgers,[4] n'ont pas observé de lésions mitochondriales avec le β-mercaptoéthanol, substance sulfhydrylée dépourvue d'action radioprotectrice, il nous a paru utile