The Histochemical Demonstration of β-D-Glucuronidase in the Reticuloendothelial System

Halogen-substituted indolyl compounds are useful histochemical substrates because the end product is intensely colored, finely particulate and insoluble. In addition, previously developed indolyl substrates for β-D-galactosidase[1], β-D-glucosidase[2], and β-D-fucosidase[3] have shown distinct specificity with respect to the configuration of the glycoside. Similar techniques with a recently synthesized compound (5-bromo-4-chloroindol-3-yl-β-D-glucopyruroniside) have been successful in detecting β-D-glucuronidase in histologic sections. This communication describes a finding of particular interest: the localization of glucuronidase activity in elements of the lymphatic system. The data on reaction specificity and tissue distribution will be described in detail elsewhere[4].

Specimens from freshly sacrificed Fischer 344 rats and New Zealand rabbits were immediately frozen in a dry ice-acetone bath; 6 micron cryostat sections were subsequently prepared. The sections were fixed in 2.5% glutaraldehyde at 4 °C for 20 min and rinsed for 5 min each in 3 changes of gum sucrose (1% gum arabic in 0.88 *M* sucrose). They were then incubated for 4 h at 37 °C in a 0.2 *M* solution buffered with NaAc at pH 4.8. In addition to 16 mg of the substrate, the solution contained 0.5 cm³ of 0.015 *M* NaCl, 0.5 cm³ of 0.015 *M* $MgCl_2$, and 8 mg of spermidine trihydrochloride. No ferro-ferricyanide was used. Following incubation the sections were dehydrated in alcohol and cleared through xylol. Occasional sections were counterstained before dehydration. A positive reaction appeared as a fine blue granular and rod-shaped precipitate.

Only a small proportion of the lymphoid cells in the adult rat thymus showed enzyme activity, and even less frequent reactions were seen in the rabbit thymus. In contrast, sections of spleen from both animals showed numerous intensely staining cells in the peripheral portions of the marginal zone (Figure 1). The lymphocytes within the central and marginal zones were unreactive. Cells lining the sinusoids in the red pulp were also positive, but less strongly reactive.

Reticular cells in the lamina propria throughout the small intestine were markedly positive in both species. In the colon the cells were both less numerous and somewhat less reactive. The mesenteric lymphoid tissue showed a particularly interesting distribution of enzyme activity. The strongest reaction was in the cytoplasm of monocytes lying free in the peripheral and medullary sinuses. Less frequent reactions were noted in cells lining the sinus and margin of the medullary cords. There was a

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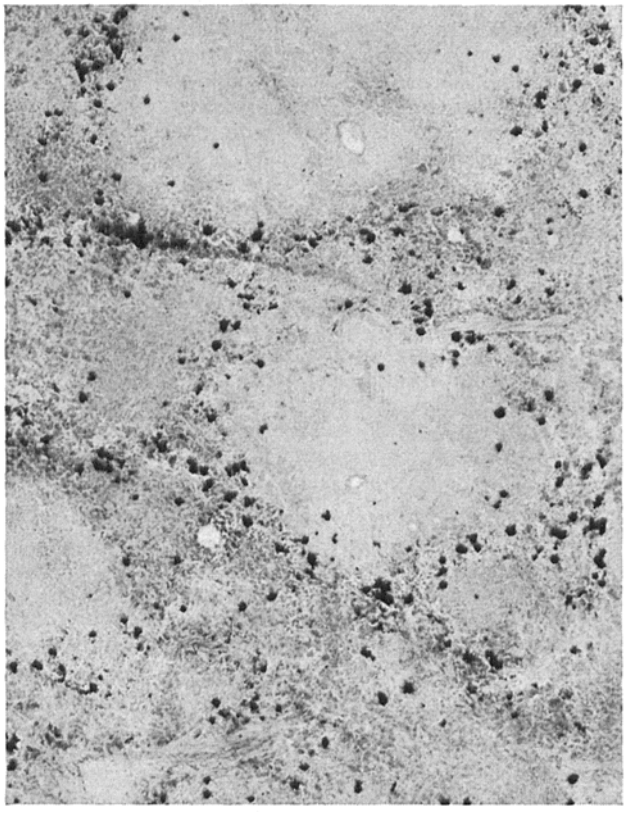

Fig. 1. Section of fresh frozen cryostat section of spleen incubated in 4-chloro-5-bromo-3-yl-β-D-glucopyruroniside. The reaction for β-glucuronidase is negative except for a few cells in the marginal zone. Reactive cells are seen on the right in cells adjacent to sinusoids. Uncounterstained, × 90.

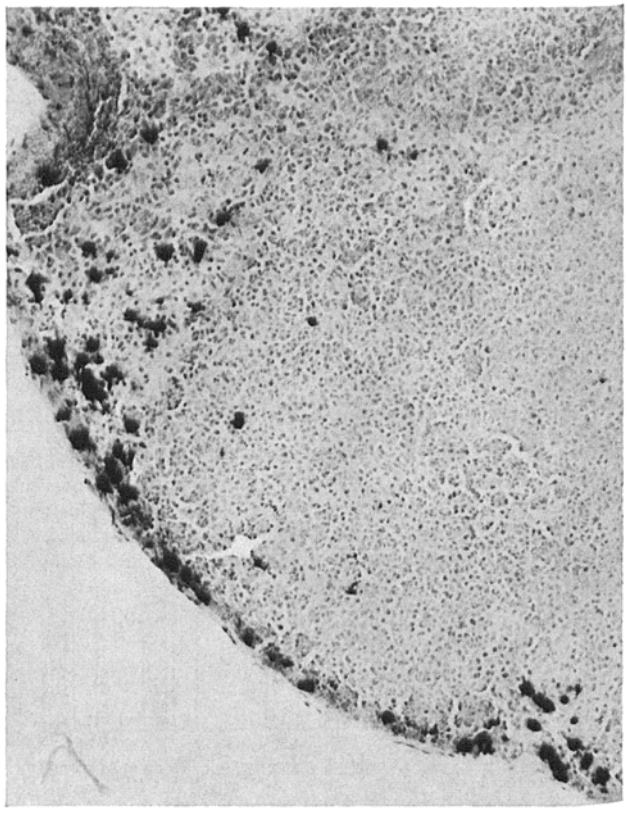

Fig. 2. A similarly incubated section of mesenteric lymph node shows numerous reactive mononuclear cells lying free in the sinusoids. The medullary cords also show a number of reactive fixed monocytic cells which aggregate at or near the littoral margins. Uncounterstained, × 145.

faint reaction in the lymphocytes in these areas as well as in cortical nodules (Figure 2). Positive reactions using this substrate have been demonstrated in rabbit alveolar macrophages and peritoneal mononuclear cell exudates[5]. Glucuronidase activity has also been found in polymorphonuclear leucocytes by a different method[6].

The results of the present study confirm the suitability of halogen-substituted indolyl substrates for the histochemical detection of enzyme activity. The reactions are characterized by satisfactory resolution, specificity, and stability. β-glucuronidase activity was found in mononuclear cells in the thymus, spleen, intestine, and lymph nodes. Reactive mononuclear cells have also been described using an analogous substrate for β-galactosidase[1,7]. These substrates provide a simple but reliable histochemical method for the study of the normal and altered reticuloendothelial system in tissue sections[8].

Zusammenfassung. Eine Halogen enthaltende Indolverbindung wurde zum Nachweis der β-D-Glucuronidase benutzt. Die Methode eignet sich zur Lokalisierung von reaktiven Kleinzellen im reticuloendothelialen System.

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Polymorphisme des lipoprotéines et des glycoprotéines sériques chez la Truite

Le polymorphisme des protéines sériques est maintenant bien établi à la suite des travaux de Béarn[1], Blumberg[2] et Hirschfeld[3].

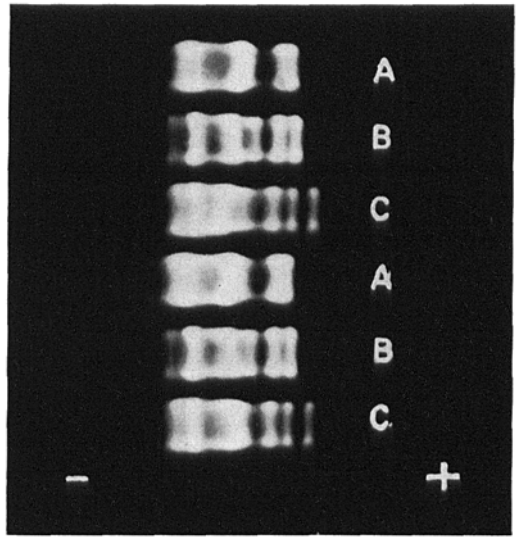
Kosswig[4] a mis le premier en évidence le polymorphisme des protéines sériques des poissons, qui a été depuis étudié par Nyman[5], Salibian[6] et Fine[7].

Nous avons étudié les sites protéiques des variations génétiques chez trois espèces de truites, soit *Salmo trutta*, *Salvelinus fontinalis*, et *Salmo gairdneri*.

Matériel et méthodes. Ces truites proviennent de la Station biologique du lac Jacques-Cartier (Québec). Elles sont gardées au laboratoire dans un aquarium contenant de l'eau douce provenant du lac St-Charles (Québec), à 4 °C, au pH de 7.6, dans un volume de 120 gallons, filtré à chaque 10 min.

Le sang est prélevé, sous anesthésie à l'uréthane, par la veine dorsale; le sérum est poolé par espèces, sans distinction de sexe, et soumis aux analyses suivantes: (a) l'analyse électrophorétique sur membrane d'acétate de cellulose à l'aide de l'appareil Spinco Duostat et sur la cellule microzone R-101. Cette analyse est faite à 250 V, 4 mA, durant 20 min, dans un tampon véronal 0.075 *M*, pH 8,6; (b) l'analyse électrophorétique a été faite sur gélose à 1,5%, en tampon véronal-barbital 0,075 *M*, pH 8,6, sur lames de microscope sur l'appareil immunophor (LKB), sous un courant électrique de 10 V/cm, durant 75 min; (c) l'analyse immunoélectrophorétique[8] a été faite sur le même appareil immunophor (LKB) en utilisant également un tampon véronal-barbital 0,075 *M*, pH 8,6 durant 75 min, sous un courant électrique de 10 V/cm.

L'anticorps nécessaire a été préparé chez le lapin, selon la méthode à l'Adjuvant complet de Freund (Difco 063859) avec, comme antigène, le sérum de *Salmo gairdneri*. Nous avons fait sur ces électrophorégrammes et immunoélectrophorégrammes, la coloration des protéines simples à l'Amidoschwartz[9], la coloration des glycoprotéines à la phénylhydrazine[10], des lipoprotéines au Soudan IV[11]. Nous avons de plus caractérisé l'activité



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