An immunohistochemical method for examining cell proliferation in the gastrointestinal tract in neonatal pigs

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The gastrointestinal tract is an organ which has very rapid cell turnover rates in its mucosal layer, involving rapid cell proliferation at the basal region and cell shedding on the luminal surface. The cellular turnover is regulated by complex mechanisms including hormones, regulatory peptides, nutrients and luminal factors. The common technique for studying mucosal cell proliferation is the autoradiography using tritium labelled thymidine. The shortcomings of this technique is the radioactivity and slow process. Here we describe a speedy immunohistochemistry method for examining cell proliferation in the gastrointestinal tract in neonatal pigs.

Bromodeoxyuridine (BrdU), an analog of thymidine, was injected intraperitoneally to suckling piglets at 30-min intervals for four times of 5 mg/kg body weight each. Thirty minutes after the last injection animals were euthanised by an overdose of thiopentone sodium. The gastrointestinal tract was immediately removed, and tissue blocks were taken and fixed in a Bouin=91s fluid for about 12 hours. The tissue blocks were then dehydrated and embedded in paraffin wax. Paraffin sections of 5 mm in thickness were dewaxed and treated with 1% bovine serum albumin to block non-specific binding sites. The sections were then incubated with mouse anti-BrdU monoclonal antibody for 60 minutes at room temperature in a humidified chamber. The antibody solution contained nuclease to expose cellular DNA for reaction with antibody. To ensure the specificity of antibody reaction several controls, eg tissue sections from animals without BrdU injection, were included. After incubation with the primary antibody tissue sections were incubated for 30 minutes with biotinylated anti-mouse Ig antibody followed by incubation for 20 minutes with peroxidase-streptavidin. All tissue sections were then incubated for about 4 minutes with 0.05% diaminobenzidine (DAB) solution. The DAB solution was prepared fresh prior to use and it was activated by addition of 12 ml of hydrogen peroxide and 1 drop of 1% cobalt chloride solution per 10 ml of DAB solution. The enzyme peroxidase polymerizes DAB in the presence of cobalt and thus stains black-brown. Cells that had incorporated BrdU were identified by the black-brown colour of their nuclei.

Using this technique we have been able to identify proliferating cells in the oesophagus, the stomach and the small and large intestines. Incorporated with an image analysis system the cell proliferation rates can be quantified. The technique has been successfully employed in our laboratory for studying the effects of dietary and oral hormone treatments on gastrointestinal mucosa growth in newborn pigs (1). The advantages of this technique are free of radioactivity and taking 2-3 hours to obtain results. The wax embedded tissue blocks and the final tissue slides can be stored for indefinite time for future examination.

1. Xu RJ, Mellor DJ, Birtles MJ, Breier BH, Gluckman PD. Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. Biol Neonate 1994;66:280-7.