

A Histochemical Study on Alkaline Phosphatase in Germ Free Mice

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In our previous histochemical study, we reported that the alkaline phosphatase (AIP) activities in the liver (bile canaliculi), spleen (perifollicular region), intestine (brush border of the epithelial cells) and lymph node (medullary cord) were extremely elevated in the germ-free mice of ICR strain compared with those of conventional mice.

In the present report, histochemical observations were made on AIP activities in the livers and the spleens of the following ICR mice: 1. Germ-free 2. Germ-free mice monocontaminated (gnotobiotics) by or immunized with (a) a single strain of enteric bacteria (cf *E. coli*), (b) *Staphylococcus aureus* (or other purulent bacteria) and (c) chrome vaccine of salmonella enteritidis. 3. conventional ICR mice with (a) no special treatment or (b) a sufficient streptomycin administration to get the enteric bacteria free state. The serum AIP titer of these groups of mice was also measured.

Eminently elevated AIP activities in the liver and spleen of Group "1" (Germ-free) mice were sharply decreased by the contamination of some enteric bacteria ("2 (a)") but not by the contamination of staphylococci ("2 (b)") or immunization with the chrome vaccine ("2 (c)").

Although the bile canaliculi (liver) and perifollicular regions (spleen) of the conventional mice ("3 (a)") showed almost no AIP activity, the intense AIP activity in the capillary endothelia remained unaltered with "the conventionalization" of germ-free mice. In the enteric bacteria-free mice, the AIP activities in the bile canaliculi and peripheral region of the splenic follicles were moderately recovered.

Changes of the serum AIP titer among these different experimental groups of mice were quite similar to those of histochemical AIP activities in the livers and spleens.

The critical decrease of the liver, spleen and serum AIP might be owing mainly to the contamination of the enteric bacteria.

The Changes of Non-specific Alkaline Phosphatase Activity in the Small Intestine of Developing Rat

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Non-specific alkaline phosphatase activity was light and electron microscopically investigated in the small intestine of rats from birth to 30 days of age. Glutaraldehyde-fixed frozen sections were incubated for 15 minutes in a mixture of 1-naphthyl

phosphate and hexazotized pararosaniline.

From the day of birth until the 15th day alkaline phosphatase activities of duodenal epithelium were observed only on membranes covering microvilli. And then, on the 23rd and 25th day, phosphatase activities were observed in the same sites as those in full grown rat, i.e. on the microvilli, the lateral cell membranes and Golgi apparatus. In the ileal epithelium, the alkaline phosphatase was localized on the microvilli and the cytoplasmic smooth membrane system until the 20th day after birth. On the 23rd day, however, the activities were no longer seen. In contrast to these changes, the localization of alkaline phosphatase in the jejunum showed no change with age: The reaction products, azo dyes, were situated only on the microvillus membrane of the absorptive cells.

**Electron Microscopic Enzyme Histochemistry on Plasma
Membrane Fraction Isolated from Rat Liver
II. Effects of Acetone upon the Activities of ATPase
and 5'-Nucleotidase of Isolated Plasma Membranes**

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Plasma membranes were isolated from rat liver by sucrose density gradient centrifugation after homogenization in 1 mM sodium bicarbonate solution. The isolated hepatic plasma membranes were treated with either absolute acetone or 10% water in acetone for 10 minutes at 25°C or 2°C prior to the incubation in the media for ATPase and 5'-nucleotidase (prepared according to Wachstein and Meisel). The treatment of the isolated plasma membranes with absolute acetone did not give any specifically inhibitory effects upon the activities of the enzymes. Ten per cent water in acetone, however, inhibited the activity of ATPase completely. The activity of 5'-nucleotidase was not significantly influenced by 10% water in acetone. The plasma membranes treated with 10% water in acetone at 2°C gave the positive reaction for ATPase activity. As previously reported the activities of both enzymes locate asymmetrically at the outer leaflets of the membrane element lining the bile canaliculi and also at the cytoplasmic surfaces of the tight junctions.

The activity of ATPase both at bile canaliculi and tight junctions showed the same behavior to the treatments with absolute acetone or 10% water in acetone as stated above. The activity of 5'-nucleotidase at both sites had the different response to the treatments from that of ATPase.

These electron microscopic findings are in good agreement with the previous light microscopic observations.

The differential effects of absolute acetone and 10% water in acetone upon ATPase and 5'-nucleotidase are explained on the basis of the phospholipid dependency of ATPase of hepatic plasma membranes.