

cytokeratin and cytokeratin 18 According to Dabike *et al* (1989) the cytokeratins are organised as a filamentous network

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Functional Specialization of the Epithelium in the Mesonephric Tubules

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Abstract. An electron microscopy study was aimed to correlate structural differentiation of the epithelium in mesonephric proximal tubules (PT) with the expression of membrane activities of alkaline phosphatase (AP) and 5'-nucleotidase (AMP) Tissue samples of mesonephros were taken from 5 to 16 days old chick embryos Both enzymes were detected with cerium technique, Mayahara modification of lead capture method was used also for localization of AP Control incubation was performed with levamisole The formation of absorptive apparatus was characterized by the differentiation of PT epithelium Activities of AP and AMP appeared to increase rapidly with the differentiation of epithelium Reaction products of AP and AMP were detected on brush border as well as on

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membranes of tubular invaginations, transport tubules and endocytotic vacuoles. The basolateral cell surfaces of epithelium were projected in short interdigitating microvilli and the expression of AP and AMP activities on their membranes suggested the transport role of this structural specialization.

Key words: Mesonephros — Chick embryos — Proximal tubule — Cytochemistry

Chick mesonephros started its function between the fourth and fifth embryonic day (ED). In our previous study we demonstrated that the development of proximal tubule (PT) preceded the differentiation of distal tubule (Jirsová and Zemanová 1999). The aim of this study was to correlate the structural differentiation of the epithelium in mesonephric PT with electron microscopical detection of membrane activities of alkaline phosphatase (AP) and 5'-nucleotidase.

Chick embryonic kidneys were taken from 5 to 16 day old White Leghorn embryos. Tissue samples were processed for electron microscopy. On selected stages of chick mesonephros development, i.e. on ED 5, 7, 9, 11, 13 and 16, two marker enzymes of cell surface membranes were demonstrated ultrastructurally. Lead capture (Mayahara *et al*, 1967) and cerium (Robinson and Karnovsky 1983) methods were used for localizing AP and 5'-nucleotidase (AMP) has also been detected with cerium technique. Tissue samples were fixed with 3% glutaraldehyde in 0.1 mol/l cacodylate buffer with 5% sucrose at pH 7.4, rinsed in cold 0.1 mol/l cacodylate buffer with sucrose, embedded in 7.5% agar and cut on a tissue chopper. Incubated sections were postfixed with a mixture of 1% osmium tetroxide and 2.5% potassium dichromate in 0.1 mol/l cacodylate buffer and embedded in Epon 812. Control sections were incubated with 2.5 mmol/l levamisole.

Primordia of PT had narrow lumen and were lined with a simple columnar epithelium. Cell apices were joined with extensive tight junctions and the luminal surface of epithelium was smooth or furnished with short rare microvilli. The intercellular spaces were narrow, of a uniform width and the basolateral cell surface had smooth course. AP and AMP activities were found only locally on membranes of the luminal cell surfaces, single microvilli and pinocytotic vesicles. The differentiation of the epithelium (ED 7) characterized by the growth of apical microvilli and formation of brush border was accompanied with increase of AP and AMP activities. Basolateral cell surfaces were projected in short microvilli which interdigitated in the wide intercellular spaces. Differentiated epithelium (ED 7-9) was absorptive type, had well developed brush border, the luminal cytoplasm showed numerous transport tubules and endocytotic vacuoles. Activities of AP and AMP were strongly expressed on the membranes of the microvilli, tubular invaginations and transport tubules (Fig 1, 3). Reaction products of AP and AMP were detected on membranes of interdigitating microvilli in the wide intercellular spaces (Fig 2). Increased activity of both enzymes was observed until ED 13.

Chick mesonephros, during its relatively short existence, represent a fully functional excretory organ. The nephrons are formed successively by the mesonephric blastema organization. The first well developed PT were found on ED 7. The early ultrastructural specialization of the epithelium was in agreement with results of our previous histochemical and electrophysiological studies (Zemanová *et al*, 1993, Zemanová and Gossrau 1996). The formation of absorptive apparatus characterized the differentiation of the epithelium in PT was accompanied with expression of AP and AMP activities. AP activity appeared to increase rapidly with differentiation of PT epithelium and local differences in the distribution of enzyme activity should be considered as a sign of different functional stage of

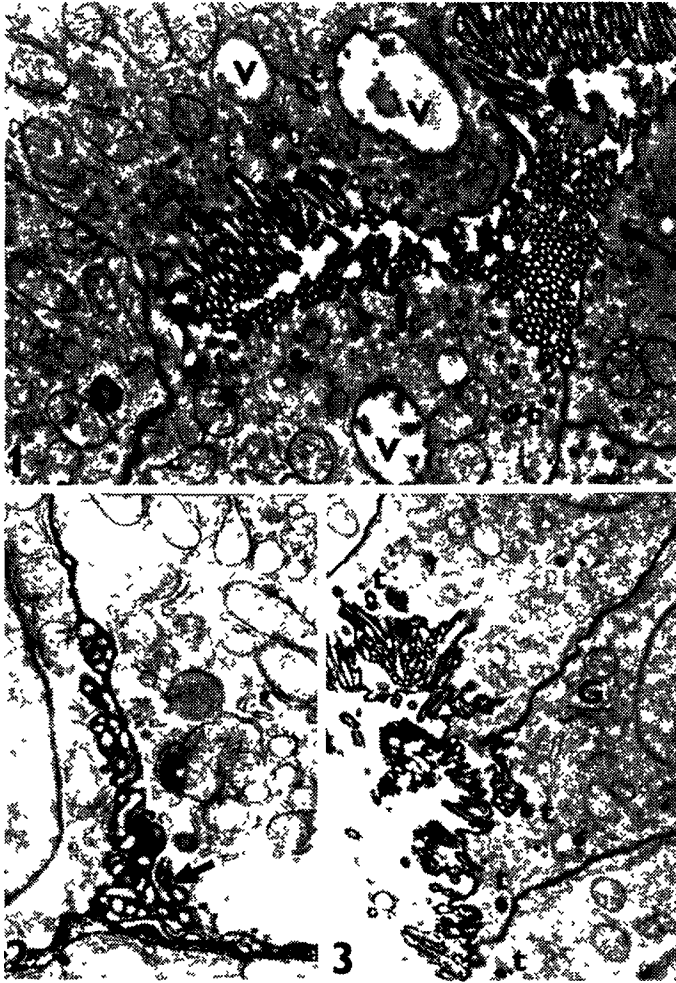


Figure 1. AP activity expressed on the membranes of microvilli, tubular invaginations and transport tubules (t) in PT epithelium ED 7 Cerium method $\times 10,760$

Figure 2. AP activity on interdigitating microvilli in the intercellular space (arrowhead) ED 9 Cerium method $\times 14,580$

Figure 3. AMP activity on membranes of microvilli and lateral cell surface of PT epithelium Reaction product expressed also on membranes of transport tubules (t) and cisternae of Golgi complex (G) ED 7 Cerium method $\times 10,500$

PT segment Results obtained with lead and cerium methods were comparable AMP is involved in transport processes and expression of its activity was used to demonstrate the transport processes in epithelium of PT Because the substrate 5'-adenosine monophosphate is also hydrolysed with non-specific AP at neutral pH, the control incubations

were performed with levamisole AMP activity was detected on brush border as well as on membranes of tubular invaginations, transport tubules and endocytotic vacuoles. The basolateral labyrinth in PT epithelium was not developed in contrary to metanephros (Narbaitz and Kacew 1978). The basolateral cell surfaces of mesonephric epithelium were projected in short interdigitating microvilli and expression of AP and AMP activities on their membranes suggested that this structural specialization should be involved in the transepithelial transport of PT.

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Total and Local Changes in the Arthritis Adjuvans

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Abstract. Arthritis adjuvans was studied in the murine model. An effect of different treatment (methotrexate, tauredon, collagen hydrolysate) was estimated in the course of developing disease (day 3, 5, 11 and 21). Repeated evaluation of body weight and peripheral blood leukograms as a total response of organism was performed. Oedema of paw, periarticular and tail regions, light- and electronmicroscopical screening and immunohistochemical investigation of prevalence of interleukin-1- β (IL-1 β) and tumour necrosis factor (TNF- α) were estimated. The most pronounced benefit effect of methotrexate at stabilization of the monocytes blood level, synovial membrane cell invasion and TNF- α immunopositivity was ascertained.

Key words: Arthritis — Synoviocytes — Macrophages — Cytokines — Therapy

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