Changes in carbohydrates on the surface of neural ectoderm during neurulation in a chick embryo

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The changes in carbohydrates on the surface of  ${\tt neural\ ectoderm\ during\ neurulation\ (from\ Stage\ 4}$ to 13) were studied by staining with ferritin-conjugated lectins or with colloidal iron (C.I.). The surface of undifferentiated ectoderm (Stage 4) was scarcely stained with RCA I, Con A, C.I. and PNA. The intensity of RCA I-staining on the cell surface increased with the progress of the development very rapidly and reached the same level as that of the neuroepithelium of neural tube (Stage 13) at the beginning of the neural plate formation (Stage 5). In contrast to the changes of RCA I-staining, the intensities of Con A-, C.I. - and PNA-staining on the cell surface were still low at Stage 5. Con Aand C.I. - staining increased at the late stage of the neural groove formation (Stage 9). PNA-staining increased at the early stage of neural tube forma-These findings suggest that the tion (Stage 13). differentiation of the carbohydrates on the surface of neural ectoderm begins at the beginning of the neural plate formation and completes by the early stage of the neural tube formation.

Regenerating Rat Livers. Shinichi Izumi\*, Mitsuyoshi Sekino\*, Tetsuya Takehiko Koji\*, Yo Chizuko Yanaihara\*\*\*, Yoshinari Moriuchi\*, Takasaki\*\*, Noboru Yanaihara\*\*\*, Kaoru Abe\*\*\* and Paul Nakane\* Cell Biol., Tokai Univ., \*Dept. Internal Med., Juntendo Univ., \*\*\*Dept. Bio-Shizuoka College of Pharm. organic Chem., Shizuoka (and \*\*\*\*Dept. Endocrinol.

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Mode of PCNA/cyclin and c-myc Antigens in

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Proliferating cell nuclear antigen (PCNA/cyclin) is an auxiliary protein of DNA polymerase delta. A domain of adenovirus E1A which has partial homology to amino acid sequences of c-myc, is known to induce the synthesis of PCNA/cyclin. In this study, we investigated the chronologic relationship between the time of appearance of c-myc and that of PCNA/cyclin in the regenerating rat livers where cells proceed from GO through G1 to S phase. The c-myc gene product and PCNA/cyclin were localized by the indirect peroxidase labeled antibody method. After the partial hepatectomy, c-myc at 12 hours and PCNA/cyclin at 21 hours were found maximally in nuclei of many hepatocytes, although neither proteins were found in normal liver. The uptake of bromodeoxy-uridine was demonstrated (at the S phase) and some mitotic figures were observed in the later time in the neclei. Accordingly, it was proved that c-myc appeared after proceeding to G1 phase and PCNA/cyclin in G1/S phase even in regenerating rat livers and is suggested that c-myc might induce the synthesis of PCNA/cyclin.

Isolaton of Carbonic Anhydrase from Human Saliva and Immunohistochemical Demonstration in salivary Glands Yoshinori KADOYA\*, Hideki KUWAHARA\*, Masayoshi SHIMAZAKI\*, Yuzo OGAWA\*\*, and Toshio YAGI\*\*. \*Second Department of Pathology, Osaka City Medical School, Osaka \*\*Department of Oral Pathology, Osaka University Dental School, Osaka

A new type of carbonic anhydrase (  $\mathsf{CA}$  ) was purified from human saliva by affinity chromatography, and antibody was produced in rabbits. The MW of the Slivary CA ( SCA ) was about 40,000. CA I,II and SCA were localized using direct immunoperoxidase method. Phenylhydrazine was used to inactivate endgenous peroxidase and to reduce nonimmunospecific binding of HRP. CAI was observed only along the blood vessels, but not in duct epithelium nor in serous acinar cells. CAII was observed in serous acinar cells, generally with more intense reaction in the basal region than in the luminal portion, and duct epithelium showed no reactivity. SCA was observed in serous acinar cells, saliva contained within the ducts, and in excretory duct epithelium. In serous acinar cells, SCA reactivity was shown to be coarsely granular and more intense toward the luminal portion. CA in saliva is possibly concerned with such activities as regulation of pH in the oral cavity and/or stabilization of supersaturated calcium phosphate in saliva secreted.

A staining method for osteocytic canaliculi in the decalcified bone tissues

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We have recently found out a staining method suitable for the osteocytic canaliculi in the decalcified The materials examined were bone bone tissues. tissues freshly obtained from rats, rabbits and humans, fixed in 10% formalin and were decalcified with neutral EDTA solution. They were then paraffin embedded by ordinary procedures. The sections sliced at 4 to 6 µm were stained by four different techniques, using picro-thionin (Schmorl 1934), silver nitrate (Christie 1977), silver ammonium (Watanabe 1959) and protargol (Bodian-Teishin 1980). The results showed that the canaliculi of osteocytes in the decalcified bone tissues were most clearly and brilliantly stained by the silver impregnation with protargol among the methods examined. By this method, even the very fine canaliculi of young osteocytes in the osteoid tissues could be stained with strong contrast, so we were easily able to identify the complicated structures of the lacuna-canaliculi system of the bone tissues. It therefore appears that this staining method would be useful for morphological analysis of the processes of bone formation and resorption using decalcified, paraffin-embedded bone tissues.