1070

Developmental Biology

DB 158

ORGAN CULTURE OF CHICK STOMACH RUDIMENTS IN VITRO. K. Takiguchi. Zool. Inst., Fac. of Sci., Univ. of Tokyo, Tokyo.

In vitro organ culture was accomplished to investigate whether the choricallantoic membrane or humorous factors derived from chick embryos are requisite for pepsinogen expression of stomach rudiments.

Proventriculi and gizzards were isolated from the 6-day chick. Liquid medium was found to be much better than agar medium with respect to the morphological and cytochemical differentiation. Mitotic index in proventricular rudiments cultured for 4 days in the liquid medium containing 50% embryo extract (EE) of 12-day embryos was lower than that in the medium containing 20% EE. However, pepsinogen expression in 50% EE was higher than that in 20% EE. In 50% EE, proventricular rudiments could express pepsinogen. Epithelial-mesenchymal recombination experiments in vitro showed that the stomach epithelia could express pepsinogen under the influence of proventricular mesenchyme, but not of gizzard mesenchyme. Proventricular rudiments could also express pepsinogen in the medium containing 10% fetal bovine serum instead of EE.

These results indicate that neither chorioallantoic membrane nor humorous factors derived from chick embryos are necessary for pepsinogen expression.

DB 159

CHARACTERIZATION OF THE cDNA FOR AN EMBRYONIC CHICKEN PEPSINOGEN. K.Hayashi¹, K.Agata², M.Mochii², S.Yasugi¹, G.Eguchi² and T.Mizuno¹. ¹Zool. Inst., Fac. of Sci., Univ. of Tokyo, Tokyo and ²Dept. of Dev. Biol., Natl. Inst. for Basic Biol., Okazaki.

Complex gland formation and expression of embryonic chiken pepsinogen(ECPg) are specific markers of differentiation of embryonic chicken proventriculus(PV). It has been known that 6 day-gizzard endoderm(GZE) or 3.5 day-allantois endoderm (ALE) form complex glands when the endoderm is combined and cultured with 6 day-PV mesenchyme on the choricallantoic membrane. In these conditions, the GZE expresses FCPg but the ALE does not

expresses ECPg, but the ALE does not. To study the molecular aspects of the expression of ECPg, we isolated the ECPgcDNA. cDNA library constructed with λ gtll expression vector was screened with mouse anti-ECPg antiserum. Consequently, we obtained cDNA 200b in length, which coded amino acid sequence highly homologous with other pepsinogens. Northern blot analysis with this cDNA as a probe revealed that ECPg-mRNA was 1.6Kb in length and was expressed maximum in the 15 day-PV. The GZE expressed ECPg-mRNA under the influence of the PV mesenchyme, while the ALE did not. This indicates that the ECPg gene could not activated in the ALE even under the influence of the PV mesenchyme.

DB 160

THE FINE STRUCTURE OF THE LARVAL STOMACH OF THE FROG, <u>RANA JAPONICA</u>. S. Ohtake¹, T. Abe¹ and N. Takeda², ¹Dept. of Biol., Nihon Univ. Sch. of Med., Tokyo and ²Dept. of Biol., Fac. of Sci. Toho Univ., Funabashi.

The feces of the tadpole of <u>R</u>. japonica fed on boiled spinach have been known to contain pheophytin. As the larval stomach secretes gastric acid, the pheophytinization is due to the acidification of chlorophyll. The present study was undertaken to clarify the fine structure of the gland cells of the larval stomach to ascertain their acid secretion.

The gland of the larval stomach consists of the secretory cells. These cells contain numerous spherical mitochondria with densely packed cristae and well developed Golgi apparatus. Zymogen granules are not observed. Elongated microvilli are the characteristics in surface structure. Sometimes a cytoplasmic invagination is found, simulating intracellular canaliculi. This invagination is lined with abundant microvilli. A number of intracellular tubulovesicular structures are located underneath the microvilli. The structure of the gland cells is similar to that of the acid secreting oxyntic cells in metamorphosed animal.

From these observations the larval stomach of <u>R. japonica</u> is ascertained histologically to have the ability of gastric acid secretion.

DB 161

MORPHOLOGICAL CHANGES OF PROPER LAYER IN VESTIBULAR MUCOSA DURING FORMATION OF MOUSE MUCOGINGIVAL JUNCTION.

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The morphological changes of the proper layer in the vestibular mucosa during formation of the mucogingival junction (MGJ) in the mouse (C3H) upper jaw were examined with the scanning electron and light microscopes. No regional difference was found on the oral epithelium during fetal stage. Only the uniformed cells which had a few microvilli were observed on the fetal oral epithelium. However, the keratohyalin granules and the horny layer which observed in the adult oral epithelium were already found in 17-day fetus. A few adult type cells appeared in the neonatal oral epithelium, but the MGJ could not identify till one week after birth. Elastic fibers were not identifiable in

till one week after birth. Elastic fibers were not identifiable in 15-day fetus, but they were already detected in the proper layer of the buccal side of the dental lamina in 17-day fetus by the method of resorcin-fuchsin staining. Elastic fibers nerve been detected in the proper layer of the palatal side. In 17-day fetus, the condensation of the mesenchymal cells was also found in the proper layer of the buccal side. The density of the mesenchymal cells reached a maximum at 17day and decreased thereafter. The density of the mesenchymal cells at the buccal side in 17-day fetus was 1.7 times more than that in the palatal side of the same fetus and was 1.9 times more than that in the buccal side of the adult.