Changes in molecular species of pepsinogens in the development of the chick[1]

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Summary. The potential peptic activity of the chick forestomach increases rapidly at the time of hatching. Together with this, the electrophoretic pattern of the pepsinogens shifts to the adult type. These changes take place even under the complete starvation of a newly hatched chick.

The allantoic endoderm of the chick and quail embryos differentiates heterotypically into the epithelium of digestive organs when it is cultivated in association with the mesenchyme of the digestive organs[2,3]. Peptic activity was induced in the allantoic endoderm combined with the mesenchyme of the forestomach, and this was substantiated by measuring the activity in the explants[4]. These facts suggest that the mesenchyme exerts an organ-specific action on the allantoic endoderm and brings about the functional and morphological differentiation of the latter. However, the nature of the pepsinogens produced in the allantoic endoderm under the influence of the mesenchyme of the forestomach has to be analyzed in relation to the normal developmental changes of the avian pepsinogens; the pepsinogen molecules of the adult avian forestomach show the plurality on polyacrylamide gel electrophoresis[5,6]. These facts led us to study the changes of the molecular species of the pepsinogens in the course of development of the forestomach. In this paper the results are presented for the total peptic activity and its electrophoretic pattern of chick forestomach from the 15-day embryo to the adult.

Material and methods. Embryos and chicks of White Leghorns *(Gallus gallus domesticus)* were used. Eggs were kept in an incubator at 38 °C. Forestomachs were homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuged at 105,000 × g for 1 h. The supernatant was used as crude extract. The potential peptic activity was assayed at 37 °C in the reaction mixture consisting of 100 µl of 10% haemoglobin (Sigma, type I), 490 µl of 0.2 M citrate buffer (pH 2.2), and 10 µl of sample. The reaction was stopped by the addition of 1 ml of 5% trichloroacetic acid (TCA). 1 unit of enzyme was defined as the amount which brings about the increase of 1 A_{280} per min in the TCA-soluble fraction under the present conditions.

For zymogram 7.5% polyacrylamide and 0.05 M Tris-acetate buffer (pH 8.2) were used. Slots of 1.0 × 0.1 cm were cut near the cathodal end of the plate (20 × 12.5 cm) and filled with 20 µl of samples containing about 0.2 mg of protein. Electrophoresis was carried out for 2 h at 30 V/cm.

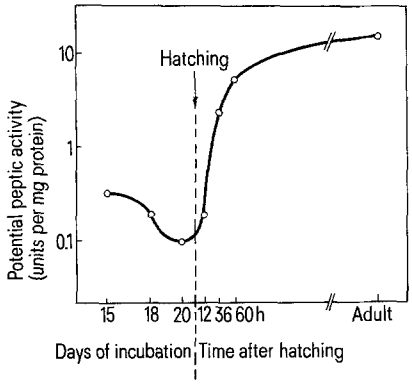

Fig. 1. The potential peptic activity of the crude extract of the chick forestomach before and after hatching. The values are the average of 2-10 forestomachs.

After electrophoresis, the gel was soaked with 0.5% haemoglobin in 0.2 M citrate buffer (pH 2.2) for 10 min, and then incubated at 37 °C for 1 h. The gel was stained with Amido Black 10B and differentiated in 7% acetic acid.

Results and discussion. The potential peptic activity in the crude extract of the embryonic forestomach expressed as units per mg protein (figure 1) increases as the development proceeds until the 15-16 day of incubation. Afterwards it decreases up to just before hatching. This decrease in specific activity is due to the rapid growth of stromal tissue of the forestomach, for the total peptic activity of the forestomach remains unchanged at these stages[7].

The peptic activity of the forestomach shows a spectacular rise after hatching. Within 24 h after hatching, the specific activity of pepsin was about 10 times that of the embryonic forestomach of the 20-day embryo and increased at the same rate in the subsequent 24 h. The activity reaches a plateau on the 3rd day after hatching. The specific activity of the adult forestomach was only about 3 times that of the 2.5-day forestomach.

The peptic activity was investigated on polyacrylamide gel after electrophoresis (figure 2). The crude extract of the adult forestomach (figure 2, f) contains at least 6 bands

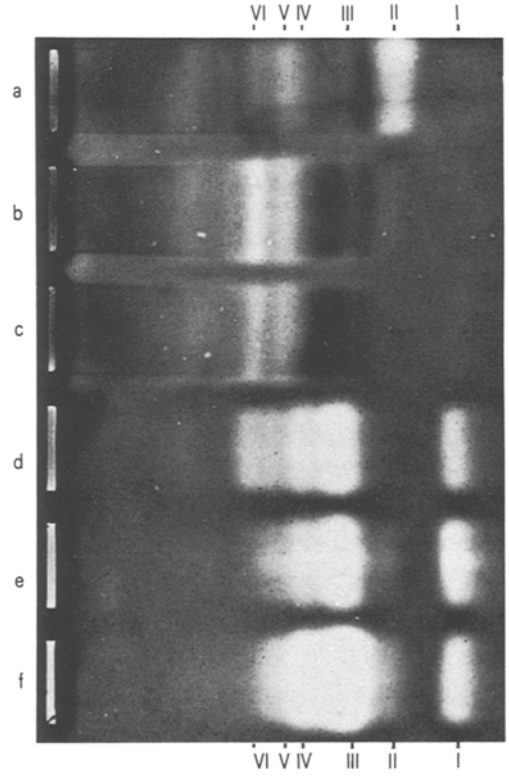

Fig. 2. Zymogram of pepsinogens of the crude extract of the chick forestomach before and after hatching. Each slot contains about 0.2 mg of protein. *a*, 15-day embryo; *b*, 20-day embryo; *c*, 12 h after hatching; *d*, 36 h after hatching; *e*, 60 h after hatching; *f*, adult.

showing peptic activity on the gel, designated as band I to band VI, with decreasing anodal mobilities. In the case of adult forestomach, band III shows the strongest activity, followed by bands IV and I, while band VI is very faint.

When pH of the crude extract of the adult forestomach was brought to 2.0 with HCl prior to the electrophoresis, all bands showed increased mobility towards the anodal end, suggesting that the materials in the bands were pepsinogens, and they gave rise to corresponding pepsins by activation.

The zymogram pattern of the crude extract of forestomach of 15-day or 20-day embryos differed from that of the adult. Band I was completely absent in 15-day extract (figure 2, a), band II being the major one. In the extract from the 20-day embryonic forestomach (figure 2, b), band I appeared in some cases, but in small quantity. Band II almost disappeared, and bands V and VI became prominent. The activity of band III was very weak in the embryonic forestomach. The disappearance of band II in the later embryonic period suggests that the material in band II is specific for the young embryonic forestomach.

After hatching, the zymogram pattern changed rapidly to the adult type, together with the rise in total peptic activity. Thus, 36 and 60 h after hatching (figure 2, d and e), the zymogram pattern was almost identical to that of the adult, though that of the 12-h chick (figure 2, c) resembled the embryonic pattern.

The possible relationship between feeding, and the change in peptic activity after hatching, was compared by the zymogram patterns of fed and unfed chicks. The zymogram patterns were completely identical between the 2 groups.

Though differences in electrophoretic mobility do not necessarily mean qualitatively different proteins, and would also include conformational changes and differences in charge, our study indicated that band I differs immunochemically from the other bands[5].

The rapid increase of the activity and changes of molecular species of digestive enzymes at the time of hatching were observed also for maltase in the chick jejunum[8]. It was suggested that these changes are regulated by hormones, especially by hydrocortisone, as hydrocortisone has an enhancing effect on the level of digestive enzymes in vivo[8] and in vitro[9,10]. In the rat stomach, the maturation of the chief cells, the increase in peptic activity and the change of molecular species of pepsinogens occur at the time of weaning[11], and these events are also regulated by hydrocortisone[12,13], suggesting that similar mechanisms are involved in the control of the digestive enzymes around the time of hatching in birds and the time of weaning in mammals. The avian pepsinogens might be a useful probe for the study of these regulatory mechanisms because of the rapid change at the time of hatching and the ease of its purification[5].

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Density gradient centrifugation of cells separated from multicellular tumor spheroids[1]

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Summary. Cells from a murine fibrosarcoma (FSa) have been grown in vitro as multicell tumor spheroids (MTS). The growth rate of these MTS was determined. Following selected periods of growth, MTS were made into a single cell suspension and separated on linear density gradients of Renografin. While only 1 population of cells was separated from small spheroids (400 µm diameter), at least 3 subpopulations of tumor cells were separated and isolated from large spheroids (800 µm in diameter).

Multicellular tumor spheroids (MTS) have been used successfully as an in vitro model of tumor growth [3]. MTS retain inherent advantages of cell culture such as simplicity, low cost, and environmental control while exhibiting cell-to-cell contact[4], chronically hypoxic cell populations[5] and gompertzian growth patterns[6]. Grdina et al.[7] have shown that density gradient centrifugation of cells separated from a solid tumor isolates multiple bands of cells which show biophysical as well as physiological differences. Density separation of exponentially growing CHO cells show only 1 density band while starved late stationary cultures have at least 3 density populations[8]. These changes suggest the influence of cell environment on the banding patterns obtained. The purpose of the present study was to determine density banding patterns in MTS during growth.

Materials and methods. A methylcholanthrene induced murine fibrosarcoma (FSa) in C3H mice was used throughout this study. In each experiment 4-6 tumors were dissected and made into single cells by the method previously described by Grdina et al.[9]. MTS were grown using the soft agar method described by Macpherson[10]. Briefly, approximately 10^6 FSa cells in 10 ml of Eagle's Basal