An Ultrastructural Study of Cutaneous Hemangioma in Two Chickens
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ABSTRACT. Cutaneous hemangioma in two laying hens was examined by light and electron microscopy. In close association with capillary and cavernous hemangioma there was a solid cell mass. Ultrastructurally, the cell mass consisted of undifferentiated mesenchymal cells and had an alveolar structure. There were transitional cell types from the alveolar structure to the capillary and cavernous hemangiomas. Hemangiomaticous structure may develop from the undifferentiated mesenchymal cells in the solid cell mass.—KEY WORDS: chicken, hemangioma, ultrastructure.

Electron microscopically, cells composing the solid cell mass varied in their size and morphology (Fig. 2). The cells were ovoid or blunt spindle-shaped, and had a large, round or elliptical clear nucleus and a prominent nucleolus. Some of them had scanty cytoplasm with few organelles, while others had abundant cytoplasm with

Hemangioma of the chicken, occurring in the skin and occasionally in internal organs, has been known for years. Histopathological description of the lesion states the presence of two forms, capillary and cavernous [2, 9]. Although viral etiology of chicken hemangioma has been established [1, 4], there seems to be no published report on electron microscopic study on it. In a previous observation made in this laboratory on cutaneous hemangioma of eight female broiler chickens at 108 days of age, we noted constantly the presence of a solid cell mass adjacent to the capillary or cavernous angiomatosus lesion (unpublished data). Endothelium forming a dense cell mass has been described in a lesion produced by inoculation of a virus [4]. This report describes the ultrastructure of the hemangioma with emphasize on the solid cell mass.

Samples were taken from two egg-laying hens, about 300 days of age. The first case (case 1) had a small lesion at the mandible and the 2nd case (case 2) had lesions at the comb and thigh. These lesions were biopsied and each specimen was cut into two parts. One part was fixed in 10% formalin solution, and paraffin sections were stained by hematoxylin and eosin. The other part was fixed in 2% glutaraldehyde, and then postfixed in 1% osmic solution. The samples were embedded in epoxy resin. Semithin sections were stained with 1% methyleneblue ethanol potassium hydroxide solution, and examined light microscopically. Ultrathin sections were stained with uranyl acetate and lead acetate and examined with a Hitachi H-800 electron microscope.

The mandibular cutaneous lesion in case 1 was a miliary nodule, reddish brown in color. A dark red-bean-sized lesion was seen at the rear edge of the comb in case 2, with a soybean sized lesion at the right lower thigh. Feathers around these skin lesions were stained with coagulated blood.

Microscopically, the lesion in case 1 consisted of a solid cell mass (Fig. 1). The cells had scanty cytoplasm and a clear round or elliptical nucleus, and arranged in structures connecting each other. The inner surface of the canaliculi was lined with cuboidal or flat endothelial cells, occasionally with blood cells in the lumens. The main lesions in case 2 were hemangiomaticous, resembling those in case 1, although they had more dilated canals showing the characteristics of cavernous hemangioma. At the peripheral area of the tumor mass, a small solid cell mass was seen.

Fig. 1. A solid cell mass adjacent to capillary hemangiomaticus lesion (upper left). Case 1. HE. × 360.

Fig. 2. Undifferentiated mesenchymal cells in solid cell mass area. Case 1. × 6,000.
considerable numbers of organelles, which included mitochondria with poorly developed cristae, centrioles, well-developed Golgi apparatus and free and bound ribosomes. These cells had neither attachment apparatus with the neighboring cells nor basement membrane around them. Interstitial elements were poor, and only small amounts of collagen fibers were observed between cells. In the solid cell mass there were cords of taller cells arranged to form a rosette-like alveolar structure (Fig. 3), with a cleft or slit-like lumen in the center. At the periphery of the solid cell mass this alveolar structure was more prominent. The nucleus of cells forming the alveolar structure lost the smooth contour and was occasionally intended. Interdigitiation or overlapping of plasma membrane with adjacent cells was observed mainly at the base of the alveolar structure. Many pinocytotic vesicles were seen near the plasma membrane (Fig. 4). The cytoplasm of alveolar cells was dark, and contained organelles such as microfilaments mainly around the nuclei and free and bound ribosomes distributed throughout the cytoplasm. Golgi apparatus and mitochondria were also seen. In addition, some of them contained lysosomes and Weibel-Palade body-like structures (W-P-like body, Fig. 5). This electron-dense rod with a limiting membrane was up to 3 μm in length and about 0.2 μm in diameter. However, we could not confirm the presence of microtubular structure inside it. A discontinuous basal lamina was observed around the alveolar structure, surrounding which electron-lucent pericyte-like cells were occasionally seen. These pericytic cells had a round or ovoid, large nucleus with fine chromatin and well developed Golgi apparatus and rough endoplasmic reticulum (RER). Some of them had well developed microfilaments arranged parallel to the plasma membrane, forming dense-body-like structure at the peripheral zone of the cytoplasm. A poorly developed basement membrane was sometimes seen close to the pericytes. The amount of collagen fiber was increased at the site where the alveolar structures had accumulated. Similarly, fibroblasts with increased RER were occasionally seen.

The hemangiomatosus region was composed of capillary structures and dendriform capillary canals (Fig. 6). Surrounding the capillary lumen were cuboid endothelial cells having indented nuclei. At the basal region of the cells, there was poorly developed interdigititation between neighboring cells and tight junction-like attaching apparatus was also seen. Cells with clear cytoplasm and rather dark cytoplasm were present. Microfilaments were located around the nucleus and many pinocytotic vesicles were seen at the periphery of the cytoplasm. W-P-like bodies were also discernible. Cytoplasmic projections into the lumen were abundant and small numbers of erythrocyotes and lymphocytes were seen in the lumen. An incontinuous basement membrane was seen at the base of the capillary structure and around the pericytes. Pericytes had abun-
dant microfilaments arranging parallel to the long axis of the cell, forming a dense body at the periphery. There was an increase in number of collagen fibers around the capillary structure and also adjacent to the pericyte-like cells. Capillary structure in some areas formed wide, diversified cavernous networks showing the characters of cavernous hemangioma (Fig. 7). Endothelial cells in these areas had very scanty cytoplasm except at the nuclear regions, and had minute cytoplasmic projections. The cytoplasm was dark, contained small numbers of free ribosomes and microfilaments, and there were numerous vacuoles of various sizes. Large spindle-shaped pericytes were located around the endothelium having a basement membrane. Some pericytes had numerous RER giving a feature of fibroblasts, and others had W-P-like bodies resembling endothelial cells. However, some of them contained abundant microfilaments and dense bodies. There were well developed collagen fibers in these areas.

Ultrastructural observation revealed a continuation of endothelial cells and pericytes comprising capillary and cavernous hemangiomatosus areas to alveolar lining cells having cuboid contour among the solid cell mass. Pinocytotic vesicles were abundant at the peripheral zone close to the cell membrane of the endothelial cells of both alveolar and hemangiomatosus lesions but no such vesicles were observed in cells composing the solid cell mass. In contrast, W-P-like bodies [14] were seen only in cuboidal endothelial cells of the alveolar structure. This observation is consistent with a view that W-P bodies appear in endothelial cells undergoing vigorous vascular neogenesis in human hemangiomas [3, 5-7, 11, 12]. W-P-like body, however, could not be observed in cells of the solid cell mass. This finding corresponds to the observation that W-P bodies develop in endothelial cells at a certain stage of angiogenesis in human brain tumors [8]. The appearance of microfilaments coincided well with the onset of polarity of endothelial cells. Neither desmosomes nor tight junction structures were observed in the present study though development of these structures has been reported in human hemangiomas [5-7, 11-13]. Similarly, development of basal membrane was rather poor in the present cases unlike a multi-layered basal membrane reported in other species [5, 6].Along with endothelial differentiation, pericyte-like cells showed diversified characteristics. In the more differentiated capillary or cavernous hemangiomatosus lesion, the pericyte-like cells had either endothelial cell-like, fibroblast-like or smooth muscle cell-like characters. Similar findings have already been reported [6, 7]. We consider that pericytes develop among the solid cell mass from cells not facing the developing vascular lumen. Regarding the origin of the pericyte, an organ culture study on human hemangioblastosma suggested that pericytes, as well as endothelial cells, differentiated from mesenchymal cells having angiogenic potential [10].

The present investigation suggests that dermal hemangioma in the chicken starts from an undifferentiated angiogenic cell mass, and undergoes differentiation from hemangoendothelioma to capillary and cavernous hemangioma.

REFERENCES