pH 7.2. Approximately 0.5 ml of this cell suspension was transferred to a Swinny adapter containing a 0.45 µm Millipore filter. Pressure was applied to a syringe filled with sucrose phosphate buffer to pack the fixed cells against the membrane. The Swinny adapter was removed and the excess sucrose buffer was withdrawn with a pipette.

Approximately 3–4 drops of sterile chick serum were layered over the packed cells. A 1.0 ml syringe containing sterile chick serum was used to force as much serum as possible through the filter. The excess serum was removed from the filter and 95% ethanol was then forced toward the filter with a syringe to form a plasma clot around the fixed, packed erythrocytes. The plasma clot attached to the filter was removed from the Swinny adapter, placed into 95% ethanol, followed by 2 changes in absolute ethanol, infiltrated with propylene oxide and Epon, and embedded in Luft's Epon mixture. Sections were made with a diamond knife on a Reichert Om-U2 ultramicrotome, post-stained with uranyl magnesium acetate and lead citrate and examined and photographed with an AEI-Corinth 275 electron microscope.

Erythrocytes obtained from patients with several other types of anemia including 5 patients with chronic erythremic myelosis (DiGuglielmo syndrome), 3 patients with severe autoimmune hemolytic anemia, 1 additional patient with severe untreated pernicious anemia, and presumed normal individuals were also fixed in acetate-buffered formalin, stained with the ammoniacal silver reagent, and examined ultrastructurally. None of these patients had Cabot rings detectable in peripheral blood erythrocytes stained with Wright's stain.

Results. Typical Cabot rings were observed in approximately one per 500 erythrocytes in films of the 2 pernicious anemia patients' peripheral bloods stained with Wright's stain (Figure 1). In their peripheral blood films stained with the ammoniacal silver reagent, 1–2 erythrocytes per 500 erythrocytes contained brown or black-staining loop or figure-eight structures closely resembling those seen in the Wright-stained specimens. Brown, orange, or black-staining granular particles often adhered to these rings or loops (Figure 2).

When ultra-thin sections of the ammoniacal silver-stained erythrocytes were viewed under the electron microscope, electron dense silver deposits resembling loops or serpentine figures were observed with a frequency approximating that seen in the peripheral blood films. Partial loops and circular patterns were also noted (Figure 3). Silver impregnation of other erythrocytes not containing the structures resembling loops or rings was not observed, and no 'matrix'-type structure or filamentous structures could be detected underlying the silver granules. No structures resembling Cabot rings or partial ring forms were observed ultrastructurally in erythrocytes from the patients with chronic erythremic myelosis, from the patient with untreated pernicious anemia whose erythrocytes did not contain Cabot rings on Wright-stained peripheral blood films, from the patients with autoimmune hemolytic anemia, or from the normal individuals.

Discussion. The present ultrastructural study employing ultra-thin sections demonstrated dense silver deposits in arrays suggesting loop or serpentine figure-eight patterns that resemble parts of Cabot rings seen in peripheral blood erythrocytes viewed by light microscopy. The binding of this histochemical stain suggests the localization of arginine-rich histone in the Cabot ring in a manner analogous to the ultrastructural localization of arginine-rich histone by the ammoniacal silver reagent in the heterochromatin of erythroblast nuclei[9]. Since it is ultrastructurally impossible to distinguish histone from hemoglobin in the erythrocyte with conventional staining methods using uranyl and lead salts, it is not surprizing that a histone component binding to the ammoniacal silver reaction product could not be visualized beneath the silver deposits.

In addition, the present studies employing uranyl magnesium acetate, a stain that is preferential for nucleic acids, could not demonstrate preferential staining of macromolecules associated with the silver deposits. Consequently, the observations described suggest that DNA is either absent from the Cabot ring or present in quantities too small to be detected by this procedure. Likewise, in the present studies, structures resembling mitotic spindle filaments could not be visualized beneath the silver deposits.

Why abnormalities of arginine-rich histone should manifest themselves in a ring-shaped structure is unknown. Arginine-rich histones are synthesized within the cytoplasm of cells on polysomes[10], and histone biosynthesis and composition are abnormal in pernicious anemia megaloblasts[11]. For reasons as yet unclear, it is possible that in severe pernicious anemia, a portion of the arginine-rich histone synthesized within the cytoplasm may not become firmly bound to DNA as it would normally, and that 'cytoplasmic currents' as described recently by Bessis[5] could cause the arginine-rich histone to condense and assume a loop or figure-eight appearance.

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On the Formation of Corpus Balbiani in the Ovogonia of Man and Guinea-Pig

Z. Takeva

Department of Anatomy, Histology and Embryology, Sofia 31 (Bulgaria), 3 October 1975.

Summary. Studies were conducted on the ovogonia of human embryos at the age of 33, 35 and 40 days. Myelin-like bodies in the nuclei and in the cytoplasm were observed. In other cases, the ovogonium contained 4–5 myelin-like bodies oriented round the centrioles. This is supposed to represent an early phase of corpus balbiani formation.

Many authors (Moore and McAlear[1], Stang-Boss[2]) claim a definite role of the nucleus in dictyosome formation. Kessel[3] asserts that the nucleus controls the morphogenesis of cytomembranes. Ruby and Webster[4] have observed a development of Golgi apparatus from myelin-like nuclear formation in bat ovogonia. Scharrer

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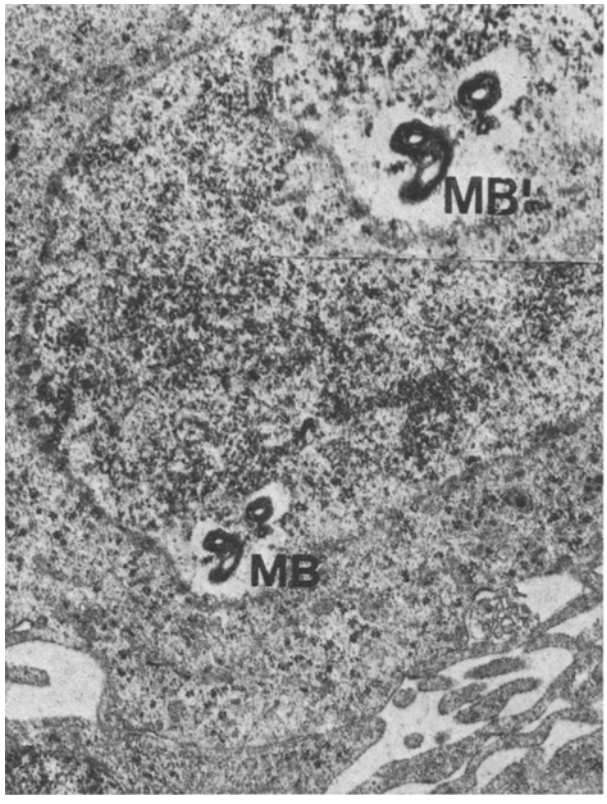

Fig. 1. Ovogonium in human embryo ovary (IV m.l.) with myelin-like body (MB) in the nucleus; ×15,000; MB', myelin-like body, ×27,000.

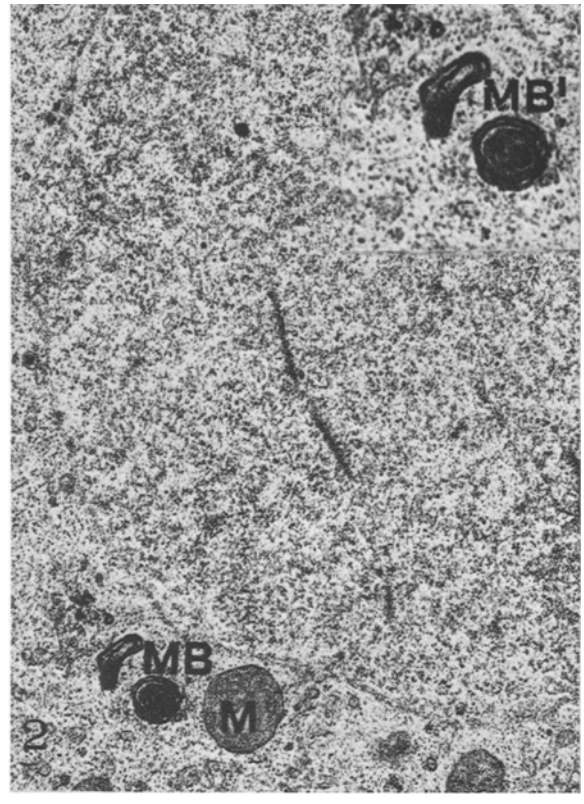

Fig. 2. Ovogonium in human embryo ovary (IV m.l.) with myelin-like body (MB) and mitochondria (M) in the cytoplasm, ×15,000; MB', myelin-like body, ×27,000.

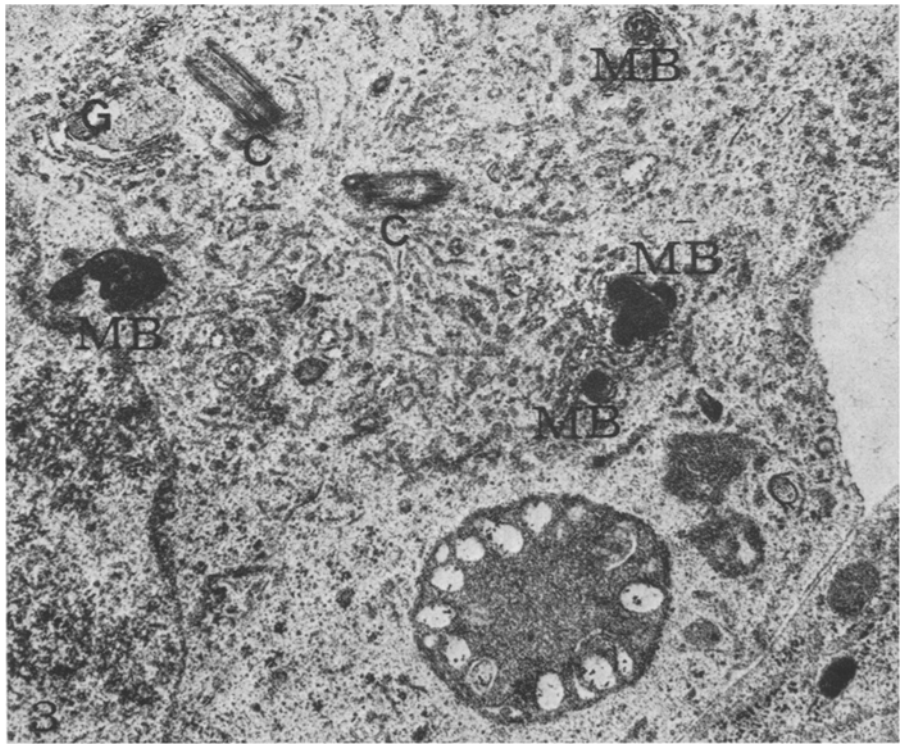

Fig. 3. Ovogonium in the human ovary (IV m.l.) with centrosomes (C), myelin-like bodies (MB) oriented around the centrosomes (C) and dictyosome (G). ×15,000.

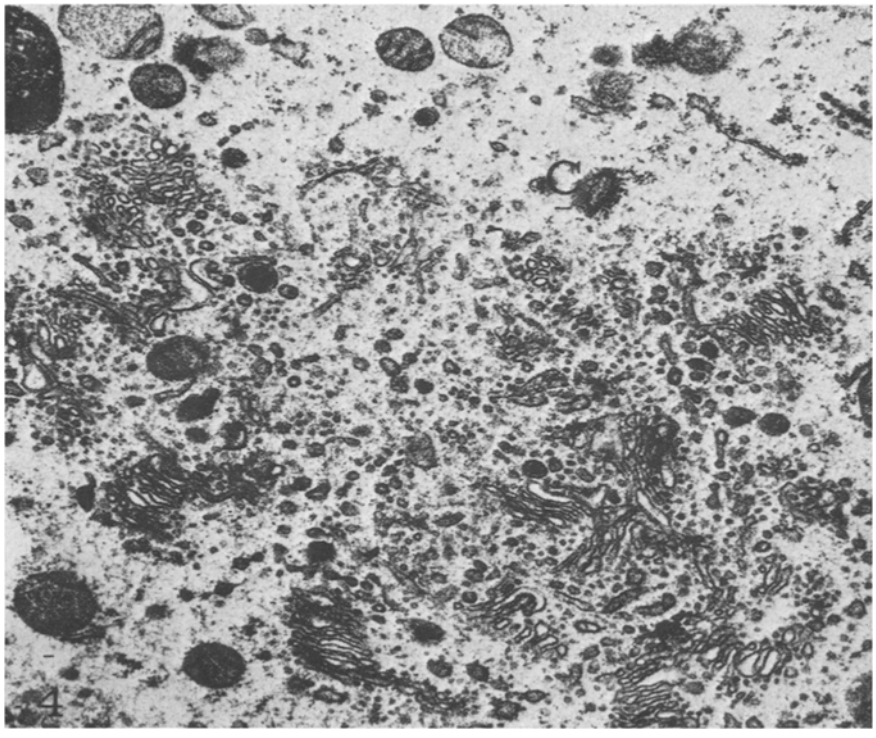

Fig. 4. A developed corpus balbiani in a guinea-pig ovocyte with centrosome (C). × 18,000.

and WURZELMANN[5] report similar observations on ovocyte of African lungfish.

The ovogonia of guinea-pig embryos (*Cavia cabaya*) at the age of 33, 35 and 40 days, as well as the ovogonia of human embryos in III and IV l.m., were studied. The ovogonia were in the prophase of the first meiotic division.

The material was fixed in precooled 4% glutaraldehyde in 0,1 *M* cacodylate (pH 7.2) buffer for 1 h, and postfixed in 1% osmium tetroxide in the same buffer for 1 h. Ovaries were parallel fixed in phosphate buffered 1% OsO_4 (MILLONIG[6]). Following dehydration and 2 changes in propylene oxide, the tissues were embedded in Durcupan ACM. The sections were cut on a Reichert OM-U_2 ultramicrotome. All sections were stained with lead acetate (MILLONIG[7]) and examined with a Hitachi Hu-11A electron microscope.

Both in man and guinea-pig, the ovogonia were clustered in cords and were always accompanied by darker cells, entirely surrounding the ovogonium. The ovogonia were light and oval. Their nuclei contained chromosomes in the prophase of the first meiotic division. Myelin-like bodies were often observed in the nucleus. In some of them, these bodies approached the nuclear membrane, which protruded at this site (Figure 1). In other ovogonia, they were observed in the cytoplasm (Figure 2).

In other cases, the ovogonium contained 4–5 myelin-like bodies oriented round the centrioles (Figure 3). Most likely this represents as early phase of corpus balbiani formation. In the ovogonia, which had already entered the last diploid phase of meiotic prophase, this structure was well developed (Figure 4).

Our observations suggest that the myelin-like formation in the ovogonia of man and guinea-pig plays some role in cell differentiation and deserves attention.

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Melanogenic Melanocytes in Human Sebaceous Glands

K. ITO[1], S. SATO, A. NISHIJIMA, K. HIRAGA, A. HIDANO[2] and A. KUKITA[3]

Department of Dermatology, Jichi Medical School, Tochigi (Japan); Department of Dermatology, Tokyo Women's Medical College, Tokyo (Japan); and Department of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo (Japan), 21 October 1975.

Summary. Electron microscopic observations revealed for the first time a small number of active melanocytes synthesizing distinctive melanin-containing organelles (melanin granules) in the ducts and acini of human sebaceous glands.

At present, elucidation of the possible distribution of melanocytes within skin appendages and of their pathogenetic significance for skin pigmentation is one of the intriguing subjects for investigation in the field of pigment cell biology. In the course of our electron microscopic study of human sebaceous glands, melanocytes actively synthesizing melanin-containing organelles were observed for the first time, which is reported in this paper.

Materials and methods. Biopsy specimens were obtained from 3 Japanese adult males and 6 newborn babies of either sex, less than 6 days of age. The materials were

[1] Dept. of Dermatology, Jichi Medical School, Tochigi, Japan.
[2] Department of Dermatology, Tokyo Women's Medical College, Tokyo, Japan.
[3] Department of Dermatology, Faculty of Medicine, University of Tokyo, Japan.