similarly fixed but processed by routine methods for TEM. Sections were cut parallel to the epiblast surface, stained with uranyl acetate and lead citrate and viewed in an AEI 801 electron microscope.

Results and discussion. In the specimens examined by SEM, long, slender bridges of the type illustrated by the authors quoted above, were observed connecting together cells of the epiblast (Figures 1 and 2). They appeared to be most numerous in stage 5 and 6 embryos, and considerably reduced in frequency by stage 9. They were much more common on the epiblast than on the hypoblast, and were not seen on the extraembryonic tissue.

The length of the bridges varied from 5 µm to a maximum of 50 µm. The very long ones had a diameter of only about 0.1 µm, and the shorter ones were up to 0.4 µm wide. Where the ends of the bridge fused with the cells giving origin to it, they were funnel shaped, but otherwise the caliber was usually fairly uniform. There was, however, a characteristic cuff-like swelling usually located about mid-way along the bridge.

The sections studied by TEM showed many microvilli projecting from the surface, and eventually sections were cut through a structure resembling the bridges seen by SEM. After more experience in studying such sections, a great many bridges could be unequivocally identified. They consisted of a narrow, linear structure containing many microtubules which were associated with dense material in the region corresponding to the location of the cuff (Figures 3, 4 and 5). Thus, the morphology of these structures corresponds exactly to that of the well-known telophase bridge composed of spindle remnant and midbody[7].

The significance of this observation is that it demonstrates a persisting bridge between daughter cells which probably remains intact long after karyokinesis is completed. Bridges often connected cells which were not adjoining each other and, in many instances which we observed, the daughter cells had obviously separated from each other a distance of several cell diameters. Presumably, the pressures created by the crowding of cells due to mitosis or migration caused the points of origin of the bridge to be moved apart. We observed bridges passing over 4 intervening cells and JACOB et al.[6] observed the bridging of 5 cells.

It would be interesting to learn whether the cells connected by the bridge give origin to the intervening cells. Possibly a second, or even third, mitosis could have taken place while the earlier bridge persisted. We attempted to investigate this possibility by searching in sectioned material for cells connected to a long bridge and also to a shorter mid-body as evidence of a second division, but we were unsuccessful. Thus, it is not possible to ascribe to the bridges any special role in intercellular communication comparable to that for cnidoblasts of Hydra or spermatids of testis, that is, a role in ensuring synchronous differentiation of clusters of cells[8]. Rather, it has come to be realized that the close junction is believed to facilitate intercellular communication, and the existence of close (and also tight) junctions in the early chick embryo has been established[9]. REVEL et al.[10] have also studied their distribution by the freeze etch method, and have concluded that junctions in the epiblast may be the source of smaller junctions later associated with migrating mesenchyme cells. On the basis of evidence presented here, the telophase bridges provide communication only between 2 cells for a maximum distance of about 50 µm.

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Ultrastructural Visualization of Cabot Rings in Pernicious Anemia[1]

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Summary. Using the ammoniacal silver stain, Cabot rings were identified in peripheral blood erythrocytes from patients with severe untreated pernicious anemia. Ultrastructural studies of these erythrocytes showed silver deposits in partial loops and figure-eight forms, indicating that arginine rich histone may be a prominent component of the Cabot ring.

Since their initial description by CABOT in 1903[2], the nature of the unusual oval or figure-eight shaped inclusions found in erythrocytes of certain patients with severe untreated pernicious anemia have been a subject of lively controversy. Although CABOT originally believed that these 'ring bodies' were nuclear remnants[2], subsequent investigators have not been able to demonstrate DNA in Cabot rings[3,4]. Some authors have suggested that Cabot rings may be remnants of mitotic spindle filaments[5], whereas others have suggested that they may be laboratory artifacts[6]. Recently, a cytochemical study of Cabot rings demonstrated that these structures possessed an unusual affinity for the ammoniacal silver reagent, and that they stained black or brown, indicating that one of their components was arginine-rich histone[7]. As a result of the ability of the reaction product of the ammoniacal silver stain to impregnate the Cabot ring and the high electron density of silver, it was possible to study this unique structure under the electron microscope.

Materials and methods. Films of capillary peripheral blood were obtained from 2 patients with severe untreated pernicious anemia. On Wright-stained coverslips viewed

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under the light microscope, many of the erythrocytes and circulating megaloblasts contained typical Cabot rings. Separate coverslip films of peripheral blood from these patients were also fixed in acetate-buffered formalin pH 7.1[8] and stained with freshly prepared ammoniacal silver reagent to demonstrate lysine-rich and arginine-rich histones[8].

Peripheral venous blood was also obtained from these patients in a heparinized Vacutainer tube. The tube containing the peripheral blood was centrifuged at 1500 rpm (450 *g*) in a Clay-Adams Safeguard angle-head centrifuge for 15 min, and the supernatant plasma was removed. The erythrocytes were washed twice in Hank's solution and fixed for 3 h in acetate-buffered formalin pH 7.1[8] at 25 °C. Ammoniacal silver staining of the erythrocytes in suspension was performed according to the method of McRAE and MEETZ[9] and the erythrocytes were suspended in a small volume of phosphate-buffered saline at pH 7.0.

The tube containing the erythrocyte suspension was centrifuged and the phosphate-buffered saline replaced with 2 changes of 0.2 *M* sucrose in 0.1 *M* phosphate buffer

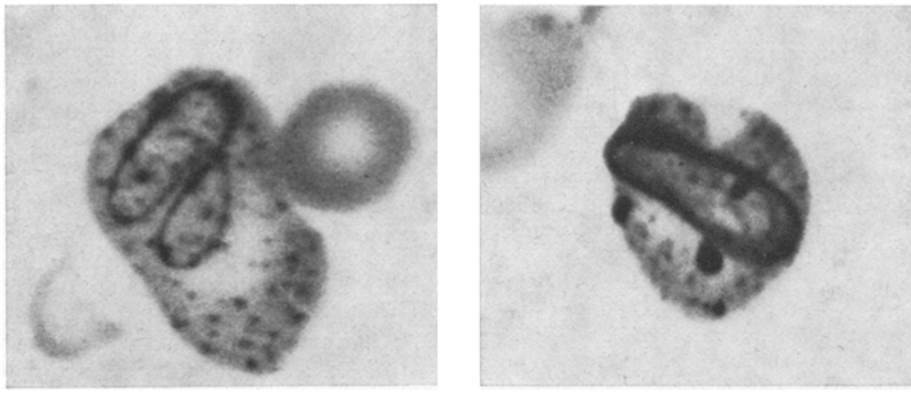

Fig. 1. Typical Cabot rings in peripheral blood erythrocytes. Wright's stain, × 1800.

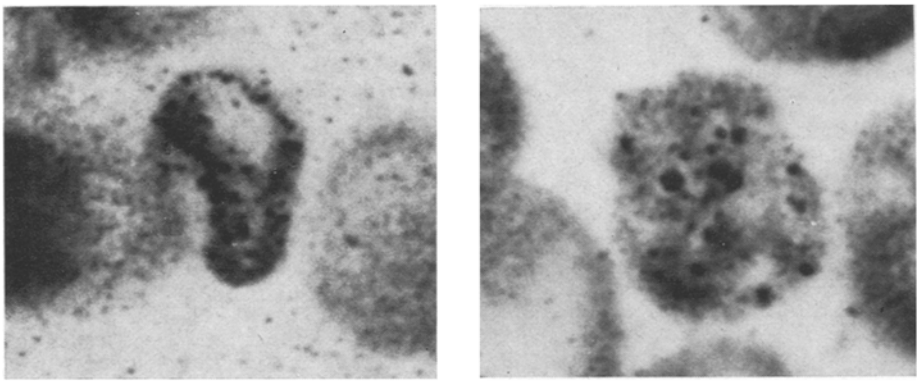

Fig. 2. Ammoniacal silver-stain of peripheral film, illustrating erythrocytes containing loop or figure-eight structures closely resembling Cabot rings visualized with Wright's stain. Brown, black, or orange particles appear to adhere to the ring, × 1800.

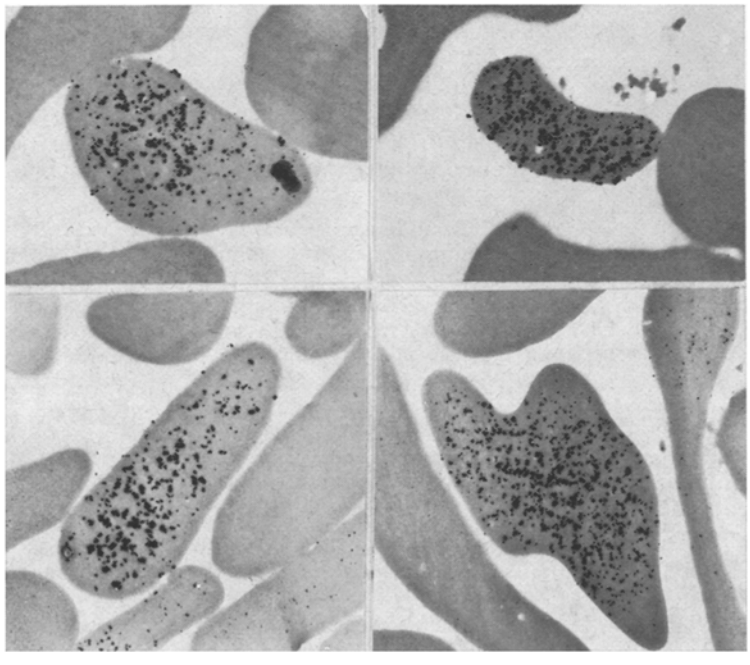

Fig. 3. Ultrastructural views of thin sections of ammoniacal silver-stained erythrocytes containing silver deposits arranged in partial loops and figure-eight patterns resembling those seen in Figure 1. Occasional random silver deposits seemingly unassociated with the partial ring or loop forms are seen in the cytoplasm of these erythrocytes, × 10,000.

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

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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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