animals were kept under the same lighting conditions. At the end of the first postnatal month, both treated and untreated rats were decapitated, the retinas of each group were dissected out and divided into 3 subgroups. The 1st subgroup retinas were processed for light microscopy (Hematoxylin and Eosin staining). The 2nd subgroup retinas were used for monoamine oxidase histochemistry[10] and the retinas after the reaction were mounted (wet mount) on the slides and observed under the light microscope to determine the intensity of the reaction. The 3rd subgroup retinas were cut into equal size pieces for biochemical rhodopsin determination[11].

Results. Histologically, no conspicuous difference was observed between the treated and untreated retinas. All the retinal layers were observed in both the control and experimental retinas. Focal areas of pyknosis, karyorrhexis, karyolysis and necrosis were absent in the nuclear layers of the treated and untreated retinas. However, the nuclear layers (expecially the outer nuclear layer) in the control retinal sections were more basophilic than the corresponding layers of the treated retinas under the routine Hematoxylin and Eosin staining. Histochemical demonstrations of monoamine oxidase (MAO) showed considerable deviation in amount between the treated and untreated retinas. There was a considerable depletion of this enzyme in the 6-hydroxydopamine-treated retinas as compared with the control retinas (Figures 1 and 2).

Biochemical determination of rhodopsin revealed a concomitant depletion of the visual pigment in the 6-hydroxydopamine-treated retinas (69.7% transmission as measured by the spectrophotometer at 505 nm) as compared with that of the control retinas (45.2% transmission as measured by the spectrophotometer).

Discussion. Our results indicated that 6-hydroxydopamine caused similar effects on the development of the visual system of the neonatal rats as that in the embryonic chick retinas[5], although the effect is less drastic in the mammalian model. The depletion of MAO in the treated retinas supports the idea there has been a decline in amount in catecholamines in those specimens. The concomitant depletion of rhodopsin in the treated retinas, on the other hand, appears to strengthen the former hypothesis that outer segment formation (i.e. rhodopsin formation at the same time) may be depended on the amount of catecholaminergic neurotransmitters.

[1] Acknowledgments. The author wishes to dedicate this paper to his family for their continuous support and to Professors A. K. S. Ho and D. B. Meyer for their understanding and friendship.

[2] H. Thoenen and J. P. Tranzer, Naumyn-Schniedebergs Arch. exp. Path. Pharmak. *261*, 271 (1968).
[3] F. E. Bloom, S. Algeri, A. Groppetti, A. Rebuelta and E. Costa, Science *166*, 1248 (1969).
[4] T. Malfors and C. Sachs, Eur. J. Pharmac. *3*, 89 (1968).
[5] D. T. Yew, A. K. S. Ho and D. B. Meyer, Experientia *30*, 1320 (1974).
[6] B. Ehinger and B. Falck, Z. Zellforsch. *100*, 364 (1969).
[7] C. W. Nichols, D. Jacobwitz and M. Hottenstein, Invest. Ophthal. *6*, 642 (1967).
[8] S. G. Kramer, A. M. Potts and Y. Mangnall, Invest. Ophthal. *10*, 438 (1971).
[9] C. R. Braekevelt and M. J. Hollenberg, Am. J. Anat. *127*, 281 (1970).
[10] A. G. E. Pearse, *Theoretical and Applied Histochemistry*, 3rd edn. (Williams and Wilkins Co., Baltimore 1972).
[11] P. Witkovsky, Vision Res. *3*, 341 (1963).

Intercellular Bridges of Chick Blastoderm Studied by Scanning and Transmission Electron Microscopy[1]

R. C. Buck, P. T. Ohara and W. H. Daniels

Department of Anatomy, Health Sciences Centre, The University of Western Ontario, London (Ontario, Canada), 16 October 1975.

Summary. Chick blastoderms were studied by scanning and transmission electron microscopy to identify by both methods a type of thread-like structure lying on the epiblast. The structure was identified by transmission microscopy as a long telophase bridge containing mid-body and spindle remnant. It apperas to provide cytoplasmic continuity between only 2 cells.

In the study of the chick blastoderm by scanning electron microscopy (SEM) a number of investigators have noted the appearance of long, thread-like cords stretching along the surface of the epiblast, and connecting together 2 cells frequently located some distance apart[2-6]. Jacob et al.[6] observed such 'connecting cords' bridging up to 5 cells and regarded them as a special form of intercellular connection. They thought it likely that the connecting cords resulted from the separation of previously contiguous cells. Bancroft and Bellairs[3] thought it possible that the threads may function to provide communication between cells that are not immediate neighbours.

Unfortunately the true identity of these structures has not been established previously because TEM micrographs were not usually prepared and those published by Bancroft and Bellairs[3] were not of adequate quality to provide clues to the identity of the structures. In light of the uncertainty about the nature of hese structures, we undertook to examine them by TEM as well as by SEM.

Methods. Chick blastoderms of Hamburger-Hamilton stages 4 to 9 were removed by a filter paper ring, fixed in 2% cacodylate buffered glutaraldehyde for 20 min, osmicated, dehydrated with ethanol, and dried by the critical point method with CO_2. After coating with silver, the specimens were examined at 20 KV in an Hitachi HHS 2R scanning microscope. Other specimens were

[1] Supported by a grant from The Medical Research Council of Canada.

[2] M. Backhouse, in *Scanning Electron Microscopy* (Ed. O. Johari and I. Corvin; IIT Research Inst., Chicago, Illinois 1974), p. 525.
[3] M. Bancroft and R. Bellairs, Cell Tissue Res. *155*, 399 (1974).
[4] J. H. Harris, Anat. Rec. *178*, 369 (1974).
[5] L. L. Litke, Anat. Rec. *181*, 411 (1975).
[6] H. J. Jacob, B. Christ, M. Jacob and G. J. Bijvank, Z. Anat. Entwickl.-Gesch. *143*, 205 (1974).

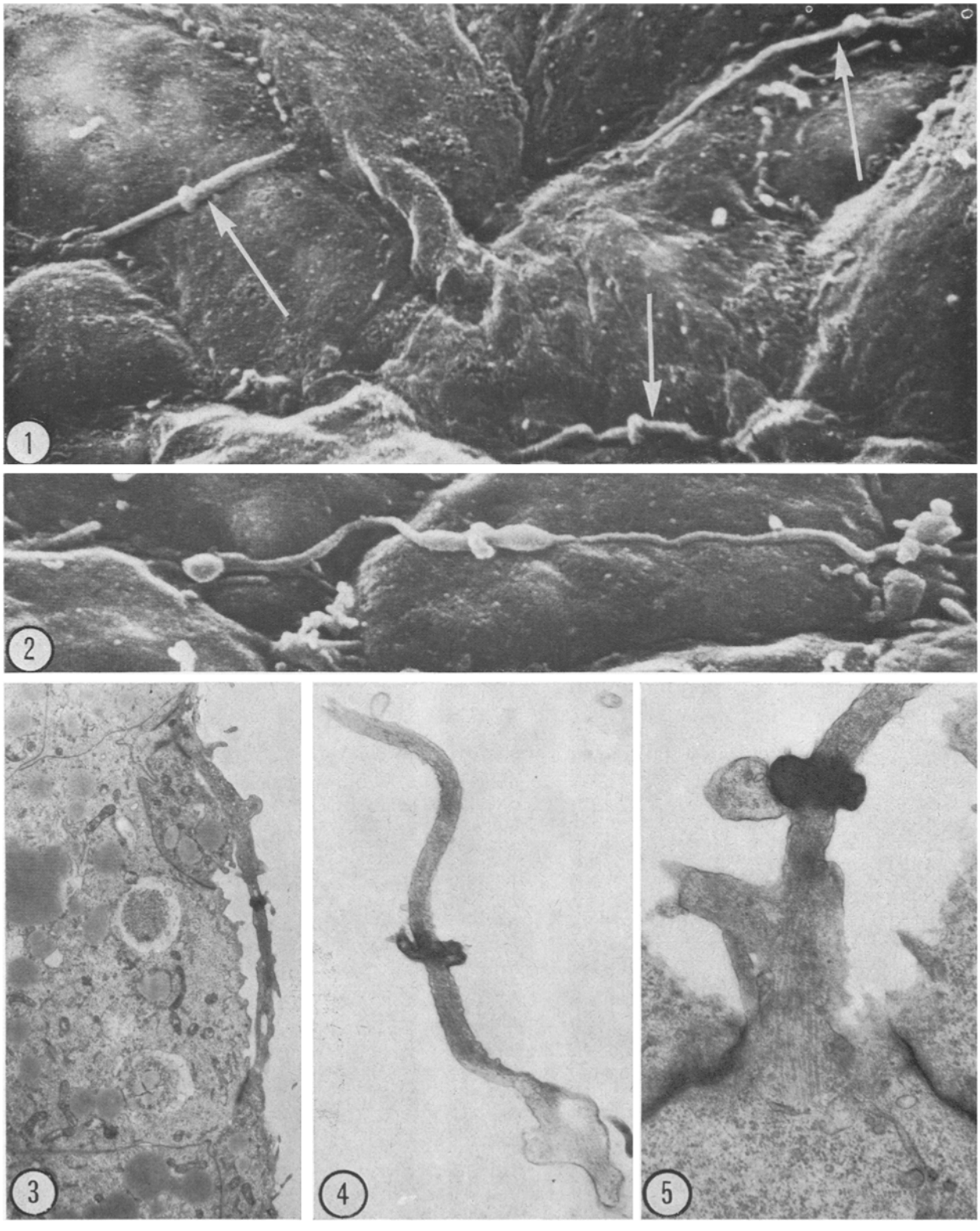

Fig. 1. SEM micrograph of epiblast showing 3 rather short bridges in this small area (arrows). Intercellular boundaries can be vaguely distinguished as rows of short microvilli. × 7,200.

Fig. 2. SEM micrograph showing a long slender bridge resting on the surface of at least 3 cells. × 12,100.

Fig. 3. TEM micrograph of a short bridge uniting cells separated by at least 1 intervening cell. Mid-body is the dense area in the bridge. × 5,000.

Fig. 4. Longitudinal section through the part of a long slender bridge which contains the mid-body. × 20,000.

Fig. 5. A bridge close to its origin including the mid-body but also showing the many microtubules of the spindle remnant. × 29,000.

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.

[7] R. C. BUCK and J. M. TISDALE, J. Cell Biol. *13*, 109 (1962).
[8] D. W. FAWCETT, Expl Cell Res., Suppl. *8*, 174 (1961).
[9] R. L. TRELSTAD, E. D. HAY and J. P. REVEL, Devel. Biol. *16*, 78 (1967).
[10] J. P. REVEL, P. YIP and L. L. CHANG, Devel. Biol. *35*, 302 (1973).

Ultrastructural Visualization of Cabot Rings in Pernicious Anemia[1]

L. KASS and R. H. GRAY

Department of Internal Medicine, Simpson Memorial Institute, Medical School, and The Cellular Chemistry Laboratory, Department of Environmental and Industrial Health, School of Public Health, The University of Michigan, Ann Arbor (Michigan 48109, USA), 10 October 1975.

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

[1] Supported in part by the Elizabeth Roodvoets Memorial Grant for Cancer Research of the American Cancer Society (No. CI-79) and by National Cancer Institute Grant USPHS No. CA 14428-02.
[2] R. C. CABOT, J. med. Res. *4*, 15 (1903).
[3] G. DISCOMBE, Revue Hémat. *9*, 173 (1954).
[4] D. PICARD, C. r. Soc. Biol., Paris *147*, 1451 (1953).
[5] M. BESSIS, *Living Blood Cells and Their Ultrastructure* (Springer, New York 1973).
[6] E. M. SCHLEICHER, J. Lab. clin. Med. *27*, 983 (1942).
[7] L. KASS, Am. J. clin. Path. *64*, 53 (1975).