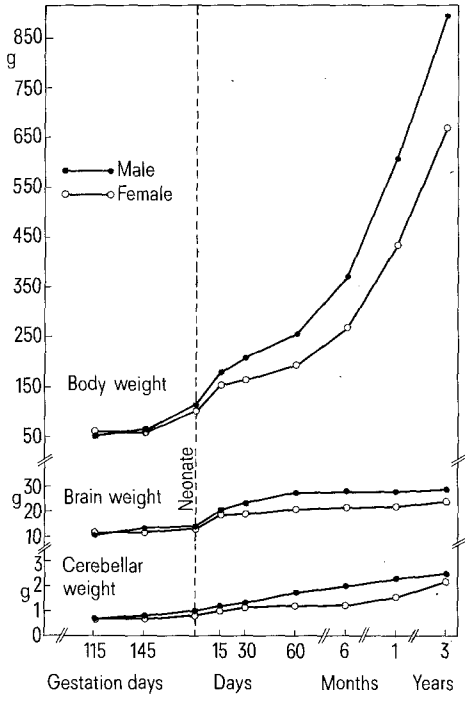
Males not only have no advantage over the females in terms of physical growth and brain development during prenatal life, but there is another interesting aspect. In our colony, maintained at about 65 animals during the breeding seasons from 1971 to the present, an average of 4 animals are stillborn and 16 are aborted each year. Out of a total number of 24 prematurely extruded fetuses, including stillbirths, 15 were males and 9 females. (Some aborted fetuses and infants born dead were cannibalized by the mothers[15].) This clearly indicates that in their prenatal life, although more males are conceived, they suffer higher rates of mortality as compared to the females. It is only in postnatal life, that they gain an edge over the females by showing accelerated rates of physical and brain growth.



1 Acknowledgments. This work was supported by USPHS Grants RR-00165 and HD-06087 from National Institutes of Health. The technical assistance of Mrs Judie Wells is greatly appreciated.
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Multinucleated cells in the retinal pigment epithelium: A scanning electron microscopic study

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Summary. Multinucleated cells in the retinal pigment epithelium of the albino rat are shown with the scanning electron microscope beside normal mononucleated cells. We suggest that such elements are formed during foetal life, owing to the absence of any mitotic activity in adult rats.

Recent studies of the ultrastructure of the retinal pigment epithelium have added much to our knowledge of its morphological features[1], its relationships with photoreceptors[2-4], and its role during the renewal of the rods[5] and, more recently, of the cones outer segments[6]. In spite of the many studies on the ultrastructural features, little attention has been paid to a characteristic detail of pigment cells, that is the observation of multinucleated elements; they were described for the first time by Ts'o and Friedman[7] with the light microscope. None of the above-mentioned authors were able to confirm such results with the transmission electron microscope; Hansson[8], with the scanning electron microscope (SEM), showed non-detailed photographs, obtained by the 'mostly possible' observation of some nuclear contours. The aim of the present study was, therefore, to show the first detailed images, obtained with the SEM, of multinucleated cells in the retinal pigment epithelium of the rat.

Materials and methods. Sprague-Dawley albino rats (200–250 g) were used and their activity rhythm (sleep-wake regimen) was synchronized by light-darkness 12:12 h, at 24±1 °C in a sound dampened room. The animals were killed by decapitation, the eyeballs rapidly excised and cut into 2 parts; the retina was picked with a pair of tweezers and then detached with a single sudden movement. The pigment epithelium was fixed in OsO_4 1% in phosphate-sucrose buffer 0.2 M at pH 7.4, dehydrated in ethanol, and then transferred to Freon 12, and finally to Freon 13 in a critical point apparatus[9]. The specimens were coated with gold and examined in a ETEC Autoscan SEM at 20 kV.

Results and discussion. The retinal pigment epithelium of the albino rat is formed by cells varying in their shape and size (figure 1). As to the shape, pentagonal (a_1), hexagonal (a_2) (both mononucleated) and heptagonal [bi- (b) or trinucleated (c)] cells are seen in a very close proximity; the hexagonal mononucleated cells have larger (5.19 µm) nuclei than the pentagonal ones (4.34 µm). As to size, next to mononucleated cells of about 12.68 µm diameter, binucleated cells of about 17.59 µm and trinucleated cells (figure 2) of about 17.82 µm diameter are seen. The

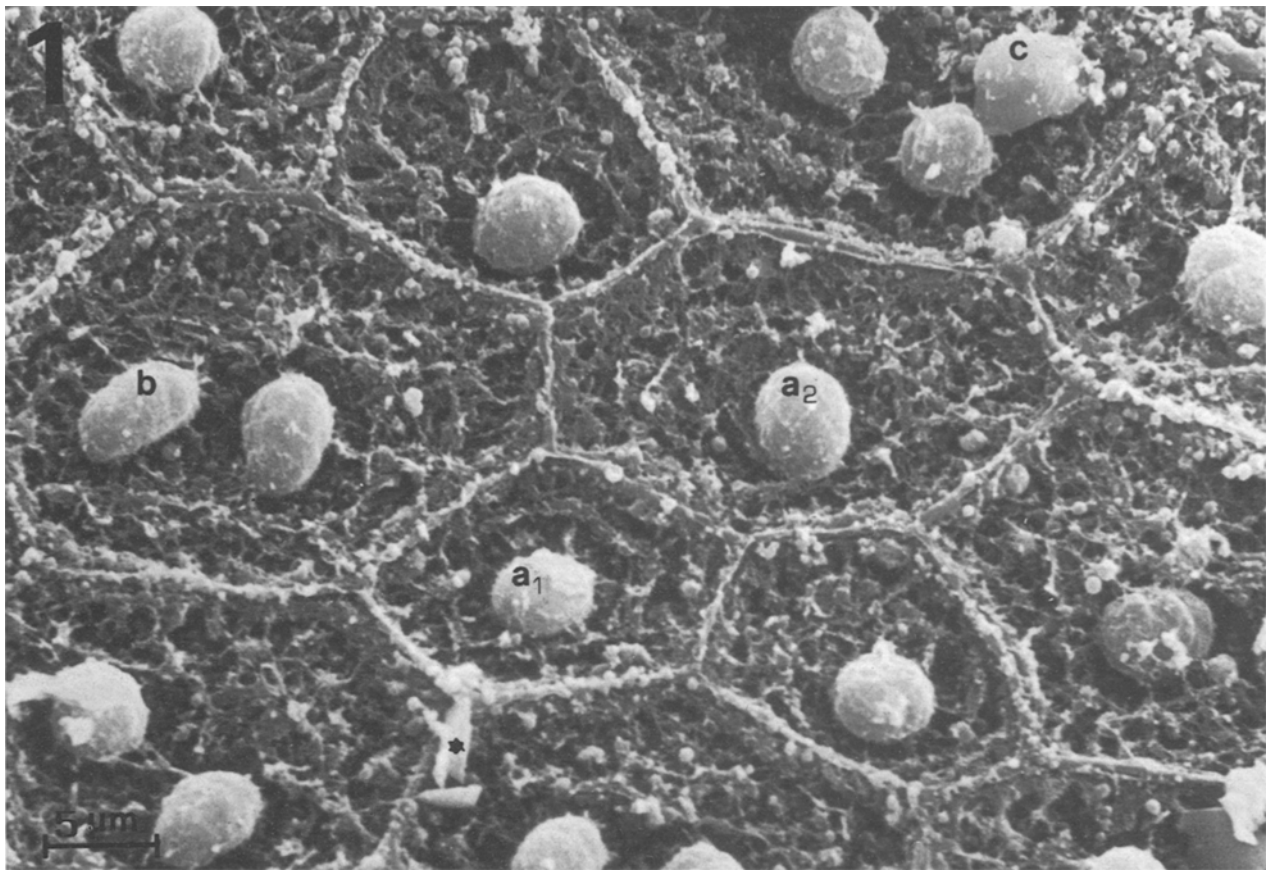

Fig. 1. Cell population in the retinal pigment epithelium of albino rat. a_1, mononucleated small cell; a_2, mononucleated large cell; b, binucleated cell; c, trinucleated cell. The asterisk (★) indicates intracellular inclusions. × 3000.

cytoplasm, when observed at a higher magnification (figure 3), is formed by an irregular granular-filamentous material, which surrounds the nucleus with a wide-mesh net; frequently, isolated or inclusions in pairs (1.8–2.1 µm in diameter) are seen. The roundish or oval nucleus shows some infoldings on its surface, to which many filamentous structures and small granules (500–1100 Å in diameter) adhere.

Up to now, the following procedures have been used to study intracellular structures with the SEM: a) collection of the back-scattered electrons from the specimen (increased volume of the tissue available for the observation, but appreciable loss of resolution)[10]; b) collection of secondary electrons from the surface of biological materials obtained by the association of mild ion-etching with low vacuum and low electrode voltage[11]; c) collection of secondary electrons from specimens which have been cryofractured[12] or cracked after embedding in styrene resins[13]. On the basis of these submicroscopical observations, the granular-filamentous material seen in the cytoplasm of the epithelial cells is undoubtely related to the organelles, which during critical point drying, gather together. Filamentous material is, most probably, formed by the lamellar, tubular and vesicular endoplasmic reticulum, whilst granular material by mitochondria, Golgi apparatus and phagosomes[6]. The phagosomes are the expression of the process of photoreceptor

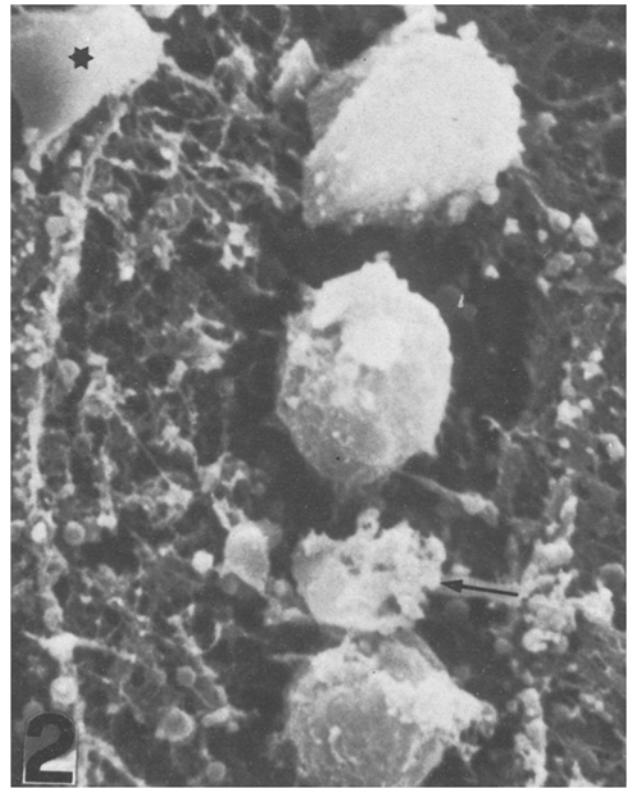

Fig. 2. Trinucleated cell. The asterisk (★) indicates an intracytoplasmatic inclusion; the arrow (→) shows a granular structure. × 8,450.

membranes replacement, which, however, before degradation, may appear free in the cytoplasm of pigment cells; we are of the opinion, on the basis of their size and of their morphology with the SEM (our unpublished results), that larger intracytoplasmatic inclusions may be packets of membranes shed from the photoreceptors.

Regarding the evidence of multinucleated cells in the pigment epithelium, Ts'o and Friedman[7], in their study of comparative histology, show that over 70% of the cells contained 2 or more nuclei; in contrast to this, on the basis of our data, obtained by young animals and by study of the whole optic cup, the frequency is about 50%. Particular attention has, furthermore, to be paid to the cell population of the pigment epithelium (table); in fact, as the nuclei increase in number, the diameter of the cells becomes wider, while the nuclear diameter reachs its maximal value in larger and mononucleated cells (5.19 µm), and then decreases in bi- and trinuclear cells (4.68 and 4.34 µm respectively). Furthermore, as the number of the nuclei and the cellular size increases, the sides of the cells are more numerous.

As to the significance of multinucleated cells, Duke-Elder and Perkins[14] suggest that they may represent an hyperplas-

Nuclear and cytoplasmatic size, number of the sides of the pigment cells

Cell	Average nuclear diameter (µm)	Average cell diameter (µm)	Number of sides
Mononucleated small	4.34	12.68	5
Mononucleated large	5.19	16.49	6
Binucleated	4.68	17.59	7
Trinucleated	4.36	17.82	7

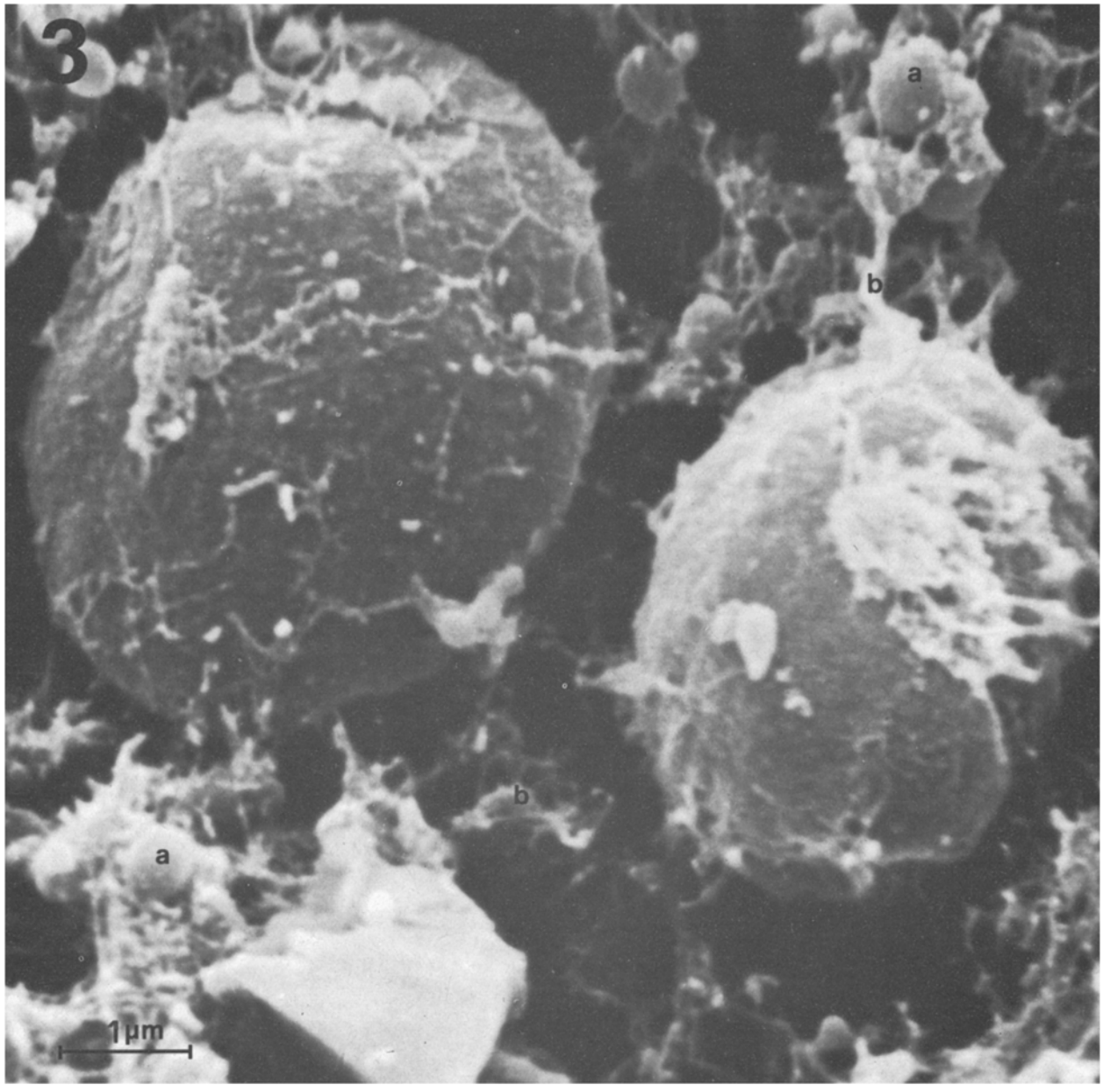

Fig. 3. Paired nuclei of a pigment cell. a, granular cytoplasmatic structures; b, filamentous cytoplasmatic structures. × 21,760.

tic senile change; whereas in accordance with Ts'o and Friedman[7], we think that they are a normal feature of the pigment epithelium, independent of the age of the animal and the region of the optic cup. The absence of mitoses, demonstrated by means of semithin serial sections, suggests that multinucleated cells may be formed during the foetal period as the result of partial and casual amitotic processes, and then persist unmodified during the life of the animals, with few and improbable changes in their number and size.

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The smooth endoplasmic reticulum as a possible storage site for dendritic dopamine in substantia nigra neurones[1]

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Summary. In dendrites of the substantia nigra neurones the monoamine marker 5-hydroxydopamine injected intracerebrally was localized inside of smooth endoplasmic reticulum cisterns. This observation opens the possibility of the existence of an alternative site for dopamine storage in dendrites as opposed to the well-known vesicular storage.

There is morphological[5,6] as well as biochemical evidence[7] that dopamine is stored in dendrites of rat substantia nigra neurones. A dopamine release from this brain area has been demonstrated in vitro[7–9] and in vivo[10,11]. Since this release is dependent on the presence of Ca^{++} in the superfusion medium[7–9] it is probable that an exocytotic mechanism is involved[12]. These observations would suggest the existence of a vesicular component for the storage and release of the dendritic dopamine. In the present study we used 5-hydroxydopamine (5-OHDA) as a monoamine marker[13,14] for the ultrastructural identification of the possible storage sites for dopamine in the dendrites of the substantia nigra neurones.

For the intranigral injections of 5-OHDA, male adult Wistar rats, weighing 280–350 g, were anaesthetized with equithesin and positioned in a Kopf stereotaxic apparatus. A 30-gauge cannula was implanted in the pars compacta of the substantia nigra in accordance with the co-ordinates from the Pellegrino and Cushman stereotaxic atlas[15]. A total volume of 1.2 µl of a 0.9% saline solution containing 120 µg of 5-OHDA and 0.1% ascorbic acid was delivered at a rate of 0.5 µl/min using a Braun perfusion pump. 40 min after the 5-OHDA injection, the animals, still under anaesthesia, were fixed by intracardiac perfusion with a solution of glutaraldehyde:formaldehyde (2.5%:2.5%) in 0.1 M phosphate buffer, pH 7.4. The brains remained overnight in 0.1 M phosphate buffer, pH 7.4, containing 5% sucrose, at 4 °C. The substantia nigra at intermediate levels from the pons to the diencephalon was dissected out from 400 µm thick slices with small knives, under direct microscopic observation. The tissue was then postfixed in 1% osmic acid in the same buffer and flat embedded in epoxy resin. Sections, 1 µm thick, were cut and stained with 1% toluidine blue for the cytoarchitectonic recognition of the substantia nigra pars compacta and pars reticulata. Areas from the medial aspects of the pars compacta and half of the adjacent pars reticulata were trimmed and selected for thin sectioning. The grids were stained with lead citrate and observed and photographed using a Philips 300 electron microscope at 60 kV.

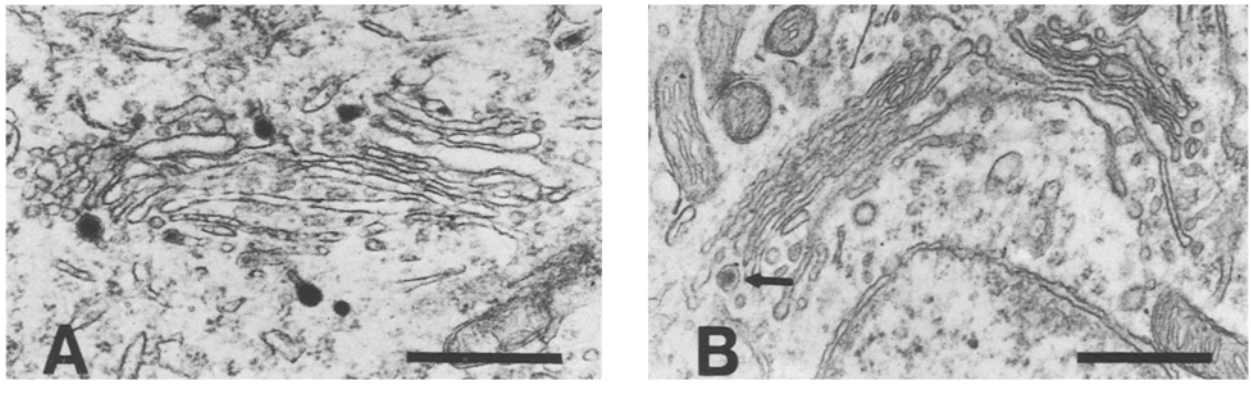

Fig. 1. *A* Electron-dense material in the interior of Golgi cisterns and vesicles of the substantia nigra pars compacta neurones following the intracerebral injection of 5-OHDA. Scale bar 500 nm. *B* Less dense material is normally present in the Golgi of neurones located in the contralateral noninjected side (arrow). Scale bar 500 nm.