JOURNAL OF ULTRASTRUCTURE RESEARCH 51, 69–78 (1975)

Intercellular Junctions in the Early Human Embryonic Retina

S. K. FISHER[1] AND K. A. LINBERG

Department of Biological Sciences, University of California, Santa Barbara, California 93106

Received July 16, 1974, and in revised form September 27, 1974; accepted October 8, 1974

Previous studies have shown that adult retinal pigment epithelial cells and early undifferentiated neural retinal cells are connected by intercellular junctions. In this study we found intercellular junctions between pigment epithelial cells, between neural retinal cells, and between these two cell types in the 28 and 36 mm human embryo. Intercellular junctions described include the zonula adhaerens, zonula occludens, macula adhaerens, and gap junctions. The gap junctions are of particular interest because of their role in intercellular communication. It appears that all cell types in the early embryonic human retina are coupled by extensive gap junctions.

In the adult human, the retina can be divided into the neural retina and the pigment epithelium. Development of the retina is a highly complex and coordinated process of cellular division, migration, and differentiation. It begins with formation of the optic pits when the human embryo is about 2.6 mm in length (refers to crown-rump length) and is not completed until several weeks after birth when the fovea has matured (*20*). The presence of pigment granules, indicating differentiation of the pigment epithelium, has been reported in human embryos as early as the 9 mm stage of development (*23*). Mann (*20*) has reported that at the 17 mm stage, differentiating ganglion cells can be found in the posterior pole of the human retina, whereas synaptic differentiation begins at the 83 mm stage (*6, 10, 28*). During early development the cells of the neural retina are separated from the cells of the pigment epithelium by the space of the optic vesicle, however these two tissues become closely apposed later (*20*) and eventually become metabolically linked in the adult (*5, 12, 22, 30*).

In the adult, the pigment epithelial cells are linked to one another by extensive junctions including gap junctions, zonula adhaerens, zonula occludens, and macula adhaerens (*13*). In the 27 to 31 mm human embryo, junctional complexes, identified as zonula adhaerens and zonula occludens, were described between pigment epithelial cells by Hollenberg and Spira (*11*); in the same tissue unspecified junctions were found between pigment epithelial and neural retinal cells. Weidman and Kuwabara (*30*) described the neuroblastic cells of the newborn rat retina as held together by well-developed junctions. They also described desmosomes which held the pigment epithelium to the neuroblastic cells prenatally but which had disappeared at the time of birth.

Because intercellular junctions, specifically the gap junctions, have been implicated in a variety of important developmental processes (*10, 27*), we have examined the retina of early human embryos for the presence of junctional complexes.

METHODS

Retinas from human embryos obtained at surgery done to interrupt pregnancy were examined by electron microscopy. This report is concerned with two embryos of 28 and 36 mm stages, or about 8 and 8.5

[1] This project was supported in part by Grant EY00888 from the U.S. Public Health Service and by a faculty research grant from the University of California. The authors wish to acknowledge the assistance of Drs. K. R. Kenyon and K. Esau, and to thank Dr. J. E. Dowling and Prof. B. B. Boycott for their critical reading of the manuscript. This project was begun at the Wilmer Institute of Johns Hopkins Hospital.

weeks of age according to the growth curves of Patten (*24*). Tissue was fixed for electron microscopy by removing the anterior portion of the eye and immersing the posterior portion in fixative. The retinas were fixed in 1.5% or 2.5% glutaraldehyde buffered to pH 7.4 with 0.06 *M* sodium cacodylate with 0.05% $CaCl_2$ and 45 mg/ml sucrose added, and embedded in Araldite. It should be noted at this time that fixation of the 28 mm stage retina in 1.5% glutaraldehyde gave superior results in this study. Thin sections for electron microscopy were obtained from one peripheral edge of the retina to the other, thus assuring that regional differences would be accounted for. All sections were cut radially through both pigment epithelium and neural retina, although the angle of section was slightly oblique. Sections were placed on copper mesh grids and stained with either 1% aqueous uranyl acetate for 20 minutes and lead citrate for 10 minutes, or in a 1:1 mixture of saturated aqueous uranyl acetate and methanol for 1 minute and lead citrate for 10 minutes. Since our tissue was not block stained in uranyl acetate, the second method was suggested to us by J.-P. Revel (personal communication) as a method which may bring out the substructure needed to identify gap junctions. Sections were examined in a Siemens Ia or 101 electron microscope.

RESULTS

The close apposition of the pigment epithelial and neural retinal cells at the 28 to 36 mm stage of development is shown in Fig. 1. The pigment epithelial cells contain dense melanin granules and their cytoplasm is much more electron dense than that of the neural retinal cells. It is apparent from Fig. 1 that both cell types contain many free ribosomes as well as some rough endoplasmic reticulum. In this region, however, pigment epithelial cells contain far fewer mitochondria than the apposing neural retinal cells. At the low magnification of Fig. 1 the high density of junctional complexes characteristic of this region can be seen.

Figure 2 shows the types of junctional complexes found between pigment epithelial cells which, with the exception of the zonula occludens, are also found within the neural retina. Four types of junctions can be seen in Fig. 2 that often, but not always, occur in complex with one another: macula adhaerens, zonula adhaerens, zonula occludens, and gap junctions.

Macula Adhaerens

The macula adhaerens (Figs. 2a and 2b; 5) found in these tissues are characterized by an increase in extracellular space to about 250 Å in width, discrete cytoplasmic plaques parallel to the inner surface of each cell membrane, and cytoplasmic filaments which converge on the plaques. In addition, the macula adhaerens contains three dense parallel lines with a diameter of about 40 Å within the expanded extracellular space giving this region a septalaminate appearance. These junctions were found commonly in the pigment epithelium, and although they were present in the neural retina, it was our impression that their frequency was fairly low. They have not been found between pigment epithelial and neural retinal cells.

Zonula Adhaerens

The most extensive junctions found in both types of tissue was the zonula adhaerens or intermediate junctions (*5*). These junctions are characterized by a slight widening of the extracellular space to about 225 Å (Figs. 2a and 6). Their most prominent feature is the dense amorphous cytoplasm which occurs along each cell membrane and contains numerous bundles of cytoplasmic filaments (Fig. 2b). The zonula adhaerens are large, often measuring 5 μm or more in length and occur within both types of tissue (Figs. 1, 2a, 5a, 6). Junctions of the adhaerens type also occurred between the two types of tissue (Fig.

FIG. 1. Low power electron micrograph of the pigment epithelium (PE) and neural retina (R) in a 28 mm human embryo. Pigment epithelial cells can be distinguished from neural retinal cells by the presence of pigment granules (P), fewer mitochondria, and a much more electron dense cytoplasm. Two types of intercellular junctions are shown: gap junctions (GJ) and zonula adhaerens (ZA). Two neural retinal cell nuclei are shown at the bottom of the figure. × 8500. Inset shows the gap junction between pigment epithelial and neural retinal cells indicated at the arrow. The difference in electron density of the cytoplasm distinguishes the two cell types. × 14 250.

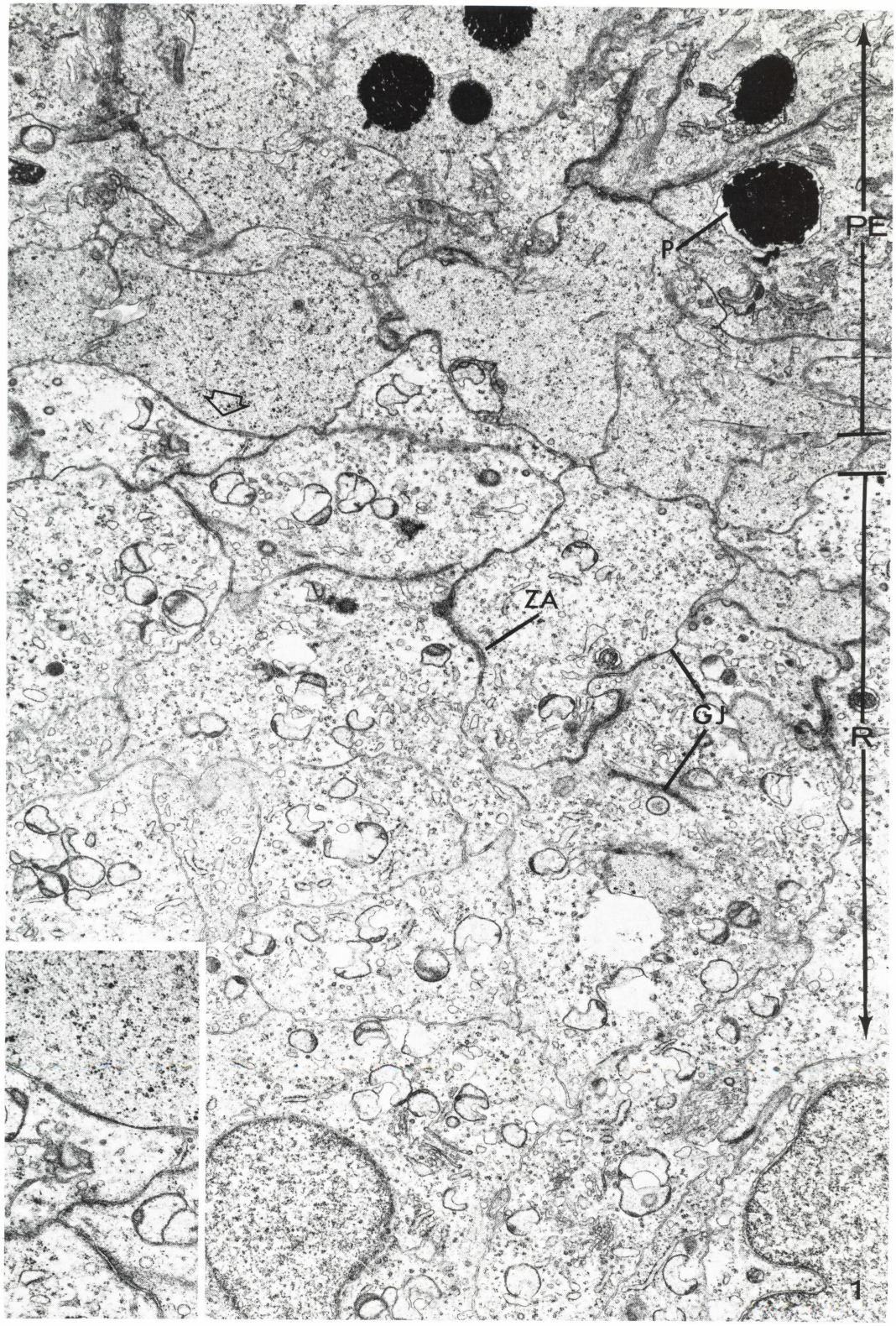


FIG. 1

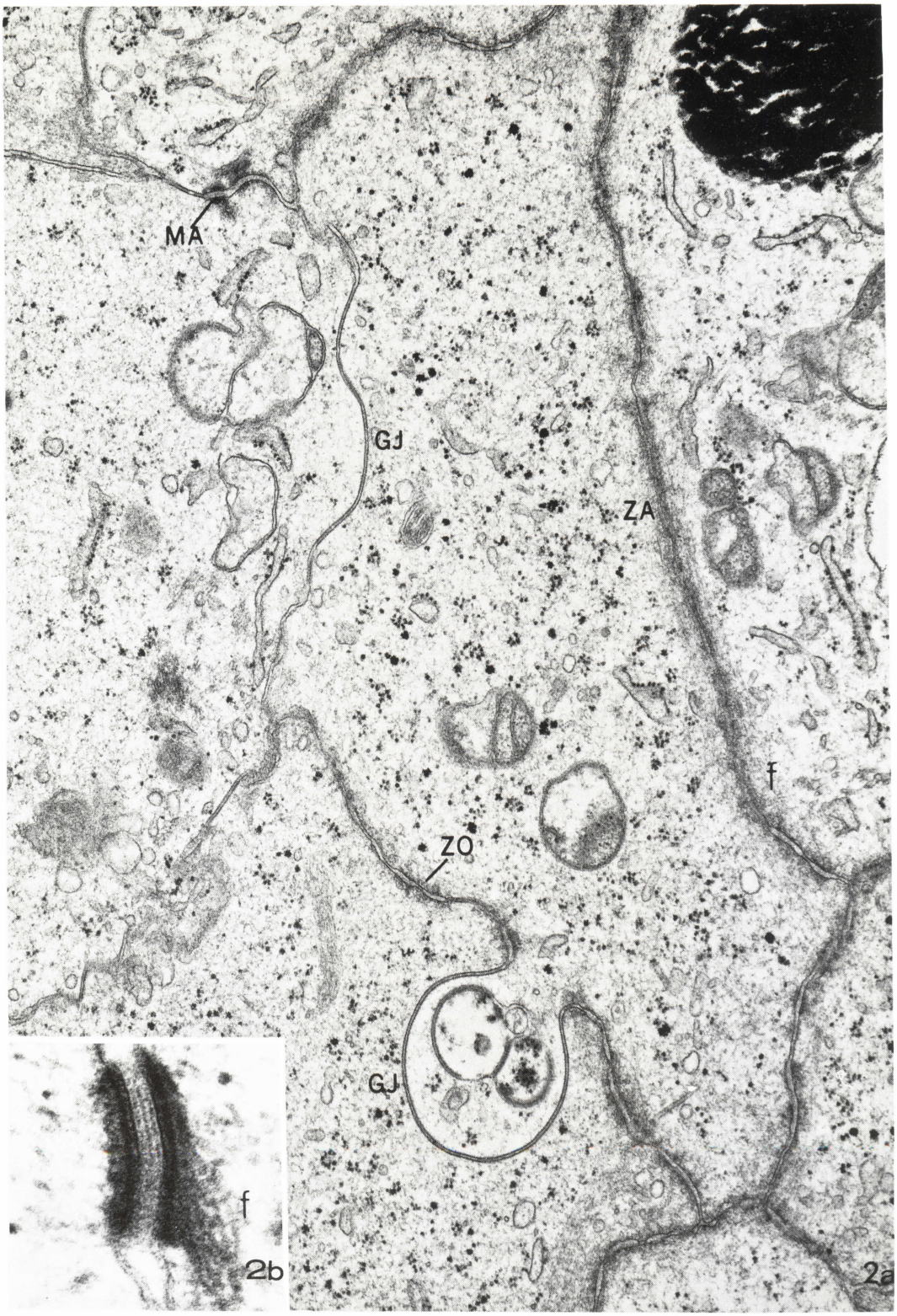


FIG. 2

4). Other cellular organelles, except for an occasional microtubule, are excluded from the space containing the filaments and dense matrix.

Zonula Occludens

Within the zonula adhaerens of the pigment epithelium small regions are found where the two cell membranes appear to come in very close apposition (Figs. 2a and 6). These points resemble the zonula occludens as described in the adult pigment epithelium by Hudspeth and Yee (*13*).

Gap Junctions

Figures 2a and 3 through 8 show regions of membrane specialization which appear to be gap junctions. These junctions are characterized by unusually dense and parallel cell membranes. At higher magnification the apparent extracellular space is occupied by a central row of dots (Fig. 4) making these junctions similar to gap junctions described by Brightman and Reese (*2*) in tissue which was not block stained with uranyl acetate. When sections were stained with methanolic uranyl acetate it was often possible to find junctions of this type which showed a central line in the extracellular space (Fig. 7). This line, having a width of 30–36 Å, is comparable to the gap width in gap junctions described in various tissues by Revel and Karnovsky (*26*), Brightman and Reese (*2*), and Hudspeth and Yee (*13*). When the junctions were sectioned *en face*, subunits characteristic of gap junctions (*13*, *26*) could often be found (Fig. 8). These junctions occur in a variety of locations, configurations, and sizes. They occur between cells of the pigment epithelium (Figs. 2a and 6), usually in association with the zonula adhaerens; between cells of the neural retina (Figs. 1, 5a, 5b, 5c, 7); and between cells of the pigment epithelium and neural retina (Figs. 1, 3a, 3b, 3c, 4). They also appear in an unusual configuration in which the cytoplasm of one cell invaginates into the cytoplasm of a neighboring cell, forming a gap junction at the invagination (Fig. 2a). This junctional configuration was commonly found in both types of tissue and was often cut in cross section yielding a circular appearance as in Figs. 1, 5b, and 6. Gap junctions were found across the entire width of the neural retina and pigment epithelium although their density was highest at the apposition of the two tissues. The length of these junctions varied widely although they generally seemed longer in

FIG. 2. (a) An electron micrograph showing the types of intercellular junctions found within the pigment epithelium. The gap junctions (GJ) often occur in a complex with the zonula adhaerens (ZA) and zonula occludens (ZO). The zonula adhaerens is accompanied by a dense, amorphous cytoplasm containing fine filaments (f). The macula adhaerens (MA) sometimes occur in conjunction with the gap junctions and zonula adhaerens. (b) Detail of a macula adhaerens in the neural retina showing the dense cytoplasmic plaques and filaments (f) adjacent to each membrane. Note the three dense lines in the intercellular space. (a) × 30 400. (b) × 152 000.

FIG. 3. Gap junctions between pigment epithelial (PE) and neural retinal (R) cells. The two cell types can be distinguished by the more electron dense cytoplasm of the pigment epithelial cells. (a, b, c) × 28 500.

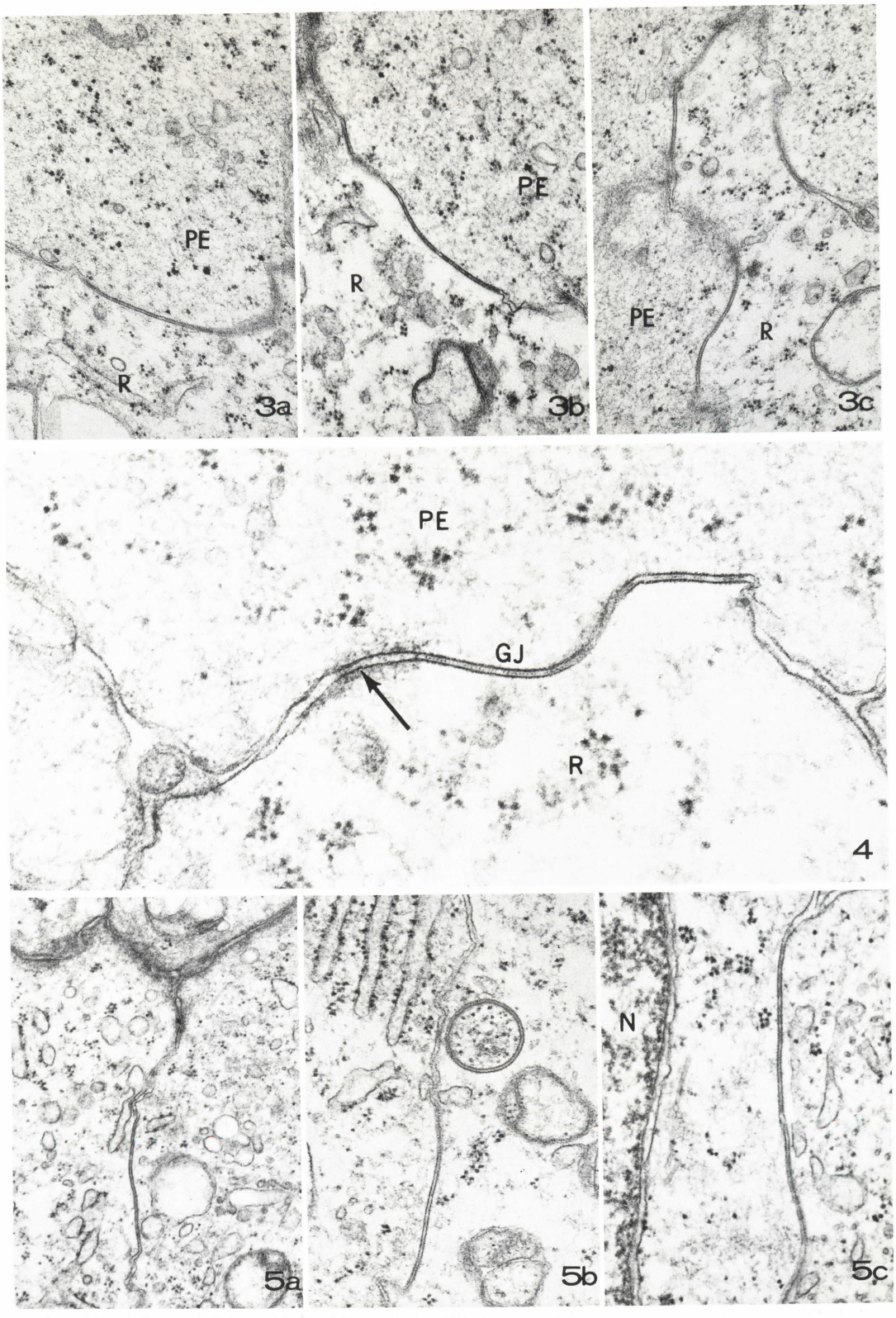
FIG. 4. An adhaerens type junction (arrow) and gap junction (GJ) between a pigment epithelial (PE) and neural retinal (R) cell. Note the series of "dots" in the intercellular space of the gap junction. × 76 000.

FIG. 5. Intercellular junctions between neural retinal cells. (a) Zonula adhaerens and gap junction near the pigment epithelium. (b) Two gap junctions, one of which encircles an invaginating process, deeper in the neural retina than (a). (c) A gap junction about midway between the inner and outer borders of the neural retina. A portion of a retinal cell nucleus (N) is shown. (a, b) × 38 000. (c) × 22 800.

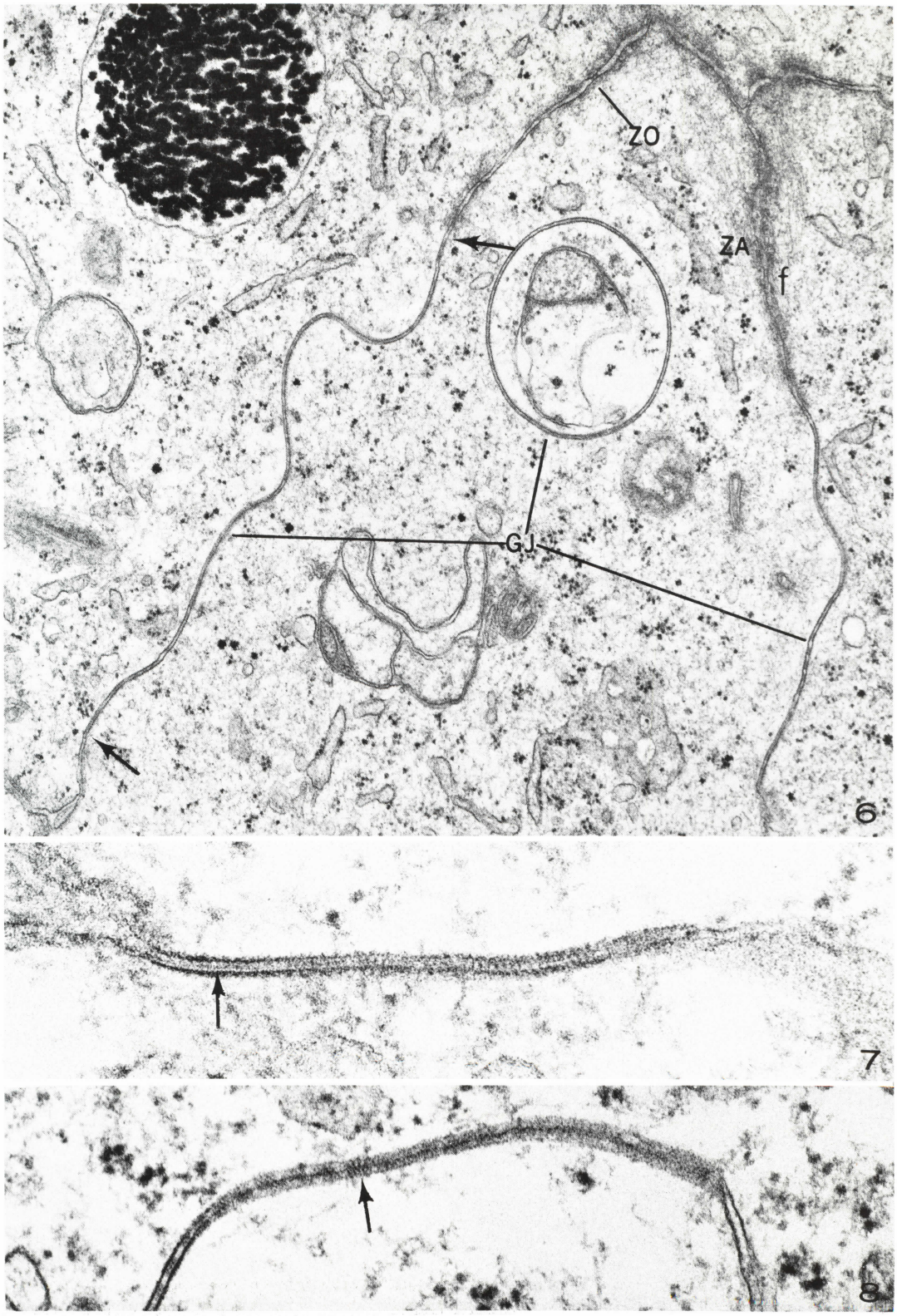
FIG. 6. Intercellular junctions between pigment epithelial cells: zonula adhaerens (ZA), zonula occludens (ZO), gap junctions (GJ). The gap junction between the arrows measures 2.8 μm in length. × 33 250.

FIG. 7. A high power electron micrograph of a gap junction after section staining with methanolic uranyl acetate. Note the line in the intercellular space (arrow). The width of the line is 30–36 Å. × 152 000.

FIG. 8. A high power electron micrograph of a gap junction after section staining with methanolic uranyl acetate. When the junction is sectioned at right angles, a row of "dots" appears in the intercellular space, when the junction begins to turn *en face* a series of subunits appears (arrow). × 95 000.



FIGS. 3–5



FIGS. 6–8

the pigment epithelium than in the neural retina. Figure 6 shows such a junction in the pigment epithelium measuring 2.8 μm in length, a typical length for junctions in this region.

DISCUSSION

The present study has shown that during early development the cells of the pigment epithelium and neural retina in man are linked by junctional complexes, some of which persist in the adult (*13*), although their form may be altered, while others disappear during development.

Our observations demonstrate the presence of the zonula adhaerens, zonula occludens, and gap junctions in the embryonic pigment epithelium which are similar to those described in adult pigment epithelium by Husdpeth and Yee (*13*) for a variety of species including the monkey. There are, however, some differences between the junctional complexes described here and those described by them. They reported that the macula adhaerens, common in other epithelia (*6*), where absent in submammalian species and rare in mammals. In the embryonic retina observed by us, the macula adhaerens were common in both pigment epithelium and neural retina. These results may reflect some difference in the embryonic and adult tissues, or a difference between man and other mammals. Hudspeth and Yee (*13*) also found that gap junctions apparently decreased in size and frequency with phylogenetic advancement so that gap junctions appeared large and frequently in thin sections of fish pigment epithelia; smaller and less frequently in amphibia and reptiles and relatively small and infrequently in mammals. The largest gap junction shown by Hudspeth and Yee (*13*, see their Fig. 12) measure slightly over 1 μm from end to end, whereas gap junctions of the size shown in Fig. 6 (2.8 μm) are common in our tissue. It was our impression that the junctions involving neural retinal cells were consistently smaller than those between pigment epithelial cells. The gap junctions shown in the vertebrate brain by Brightman and Reese (*2*), in various pigment epithelia by Hudspeth and Yee (*13*), and those observed by Dixon and Cronly-Dillon (*4*) in the developing *Xenopus* retina always occur as two parallel membranes in either a straight or gently curved line. In the embryonic human retina these junctions are often found completely encircling what appears to be a small region of invaginating cytoplasm from an adjacent cell. These unique junctions may represent a means of increasing the area of cell contact. Drawings of undifferentiated neuroepithelial germinal cells which are impregnated by the Golgi technique in the developing rat retina by Morest (*21*) show many small spinelike processes extending from the main body of the cells. We have observed similar small processes in Golgi impregnated neuroepithelial germinal cells in the embryonic human retina (Fisher, unpublished). These small, spinelike processes may be invaginating the cytoplasm of adjacent cells and forming the unusual gap junctions observed.

Gap junctions have been implicated in a variety of physiological processes including many which are potentially important to cellular development and differentiation (*1*, *7*, *10*, *18*, *25*). Gilula *et al.* (*9*) have shown that cultured fibroblasts of different types metabolically interact only when they are ionically coupled, their study showing the ionic coupling was always accompanied by gap junctions between the cells. Thus it seems likely that the extensive array of gap junctions found in the present study indicates some form of coupling between the differentiating retinal cells.

It is apparently important during development to maintain contact between neural retina and pigment epithelium (*3*, *29*) to prevent the formation of ectopic neural retina by pigment epithelium. Junctions between these two tissues have been described previously in developing rat (*30*)

and human (*28*) retinas. Wiedman and Kuwabara (*30*) described them as desmosomes, whereas Hollenberg and Spira (*28*) described them as zonula adhaerens. Our results show that the two tissues are coupled by both gap and adhaerens type junctions. Although we did not study the latter in serial sections, they appear identical to those described by Hollenberg and Spira (*28*). It seems plausible that the two tissues may be held in close apposition by the zonula adhaerens (*11*), while molecular information may pass between them through the gap junctions. At some unknown time during development these junctions are lost between the two types of tissue yet they retain important metabolic coupling in the adult.

Dixon and Cronly-Dillon (*4*) showed the presence of specialized junctions, which they assumed to be gap junctions, between retinal cells of the *Xenopus* embryo. These junctions were reported to disappear just before the presumed time of ganglion cell specification (*14*, *15*), and a functional correlation between the two events was suggested. If we assume that an analogous specification of ganglion cells occurs in the human retina just before ganglion cell differentiation, it would be difficult to suppose a similar correlation inasmuch as Mann (*20*) reported that the first ganglion cells migrate to the vitreal border in the posterior retina as early as the 17 mm stage of development. Gap junctions were found in both posterior and peripheral retina in the present study as late as the 36 mm stage.

The zonula adhaerens and zonula occludens observed by us are essentially the same as those described by Hudspeth and Yee (*13*), and a similar function can be hypothesized: namely, an adhesive junction for the zonula adhaerens (*2*, *7*) and a sealing of the intercellular space for the zonula occludens (*13*).

The macula adhaerens found in this study differ somewhat from those described by Farquhar and Palade (*6*) in various epithelia, and by Kelly (*17*) in developing epidermis, in that the intercellular cleft in our material shows three distinct dense lines, each about 40 Å in diameter, whereas one dense central disc was reported for their material.

It may be significant that only the adhaerens type and gap junctions occur commonly between cells of neural retina and pigment epithelium and that the zonula occludens and macula adhaerens with associated filaments and extracellular periodic lines were never observed in this location.

It seems likely that the extensive junctions found in the early developing retina play an important role in the developmental process. The physiological significance of the junctions will necessarily have to await appropriate data.

REFERENCES

1. Azarnia, R., Larsen, W. J., and Loewenstein, W. R., *Proc. Nat. Acad. Sci. USA* **71,** 880 (1974).
2. Brightman, M. W., and Reese, T. S., *J. Cell Biol.* **40,** 648 (1969).
3. Coulombre, J. L., and Coulombre, A. J., *Develop. Biol.* **12,** 79 (1965).
4. Dixon, J. S., and Cronly-Dillon, J. R., *J. Embryol. Exp. Morpho.* **28,** 659 (1972).
5. Dowling, J. E., *Nature (London)* **188,** 114 (1960).
6. Farquhar, M. G., and Palade, G. E., *J. Cell Biol.* **17,** 375 (1963).
7. Fisher, S. K., *Assoc. Res. Vision Ophthal.*, Spring Meeting, Abs. 50 (1973).
8. Furshpan, E. J., and Potter, D. D., *in* Moscona, A. A., and Monray, A. (Eds.), Current Topics in Developmental Biology, Vol. III, p. 95. Academic Press, New York, 1968.
9. Gilula, N. B., Reeves, O. R., and Steinbach, A., *Nature (London)* **235,** 262 (1972).
10. Gilula, N. B., *Amer. Zool.* **13,** 1109 (1973).
11. Hollenberg, M. J., and Spira, A. W., *Amer. J. Anat.* **137,** 357 (1973).
12. Hubbard, R., *J. Gen. Physiol.* **39,** 935 (1956).
13. Hudspeth, A. J., and Yee, A. G., *Invest. Ophthalmol.* **12,** 354 (1973).
14. Jacobson, M., *Develop. Biol.* **17,** 202 (1968).
15. Jacobson, M., *Develop. Biol.* **17,** 219 (1968).
16. Kanno, Y., and Lowenstein, W. R., *Nature (London)* **212,** 629 (1966).
17. Kelly, D. E., *J. Cell Biol.* **28,** 51 (1966).
18. Loewenstein, W. R., *Ann. N. Y. Acad. Sci.* **137,** 441 (1966).

19. LOEWENSTEIN, W. R., *Develop. Biol., Suppl.* **2,** 151 (1968).
20. MANN, I., The Development of the Human Eye. Grune & Stratton, New York, 1969.
21. MOREST, D. K., *Z. Anat. Entwickl-Gesch.* **131,** 45 (1970).
22. NOELL, W. K., *J. Opt. Soc. Amer.* **53,** 36 (1963).
23. O'RAHILLY, R., *Contr. Embry. Carnegie Inst., Wash.* **38,** 1 (1966).
24. PATTEN, B. M., Human Embryology, p. 173. McGraw-Hill, New York, 1953.
25. PAYTON, P. W., BENNETT, M. V. L., AND PAPPAS, G. D., *Science* **166,** 1641 (1969).
26. REVEL, J.-P., and Karnovsky, M. J., *J. Cell Biol.* **33,** C7 (1967).
27. SHERIDAN, J. D., *Amer. Zool.* **13,** 1119 (1973).
28. SPIRA, A. W., AND HOLLENBERG, M. J., *Develop. Biol.* **31,** 1 (1973).
29. STONE, L. S., *Ann. N. Y. Acad. Sci.* **49,** 856 (1948).
30. WIEDMAN, T. A., AND KUWABARA, T., *Invest. Ophthalmol.* **79,** 470 (1968).
31. YOUNG, R. W., AND BOK, D., *J. Cell Biol.* **42,** 392 (1969).