Perisinusoidal Cells in a Three-Dimensional Organization of the Adrenal Cortex in the Monkey*

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Summary. The adrenal cortex in the macaque monkey was examined under the SEM and TEM to elucidate the surface fine structure of perisinusoidal cells in a three-dimensional organization of the cortex.

Parenchymal cells in polyhedral form were arranged in acini in the zona glomerulosa, or in the laminae of closely stacked cells in the zona fasciculata and reticularis. Those acini and laminae were accompanied by sinusoids lined only with an endothelium. Endothelial cells were composed of a protruded perikaryon and a thin layer of peripheral cytoplasm in sieve plates with numerous fenestrations. The perikaryon was sometimes equipped with groups of fenestrated disc-like protrusions. Fine collagen fibrils were distributed in the perisinusoidal space, which was a continuous, slit-like lacune between the endothelial and parenchymal cells.

In the perisinusoidal space or intercellular spaces among parenchymal cells were seen two types of perisinusoidal cells: the stellate interstitial cells with several spiny, or attenuated processes entangled with collagen fibrils, and the monstrous wandering cells usually associated with pseudopodia-like processes. The cytoplasm of the interstitial cells showed a structure similar to that of fibroblasts, and contained small numbers of lipid droplets. Intravenously injected chicken blood cells were phagocytized by macrophages on the sinusoidal lining. The fine cytological structure of the macrophages was almost the same as that of the wandering cells. The present findings suggest that the interstitial cells are fibroblastic in nature and belong to the vitamin-A storing cell system, and that the wandering cells are macrophages derived from hematogenous monocytes.

The three-dimensional architecture of the adrenal cortex has attracted uncommon attention with special reference to the correlation between the fine structure of the sinusoidal capillary and the endocrine function of the parenchymal cells. This is because the parenchymal cells are in a specific zonal arrangement bestowed with the function of cell maintenance in the cortex (IDELMAN, 1970; LONG, 1975; NUSSDORFER, 1980) and specialized hormone production (NUSSDORFER, MAZZOCCI and MENEGHELLI, 1978; NUSSDORFER, 1980), whereas the endothelial lining of the sinusoid is engaged not only in hormone transport (LONG and JONES, 1967; LUSE, 1967), but reportedly also in phagocytosis (STUART, 1970; CARR, 1973).

Adding to the light microscopic data from ELIAS and PAULY (1956), and PAULY

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(1957), Motta, Muto and Fujita's (1979) recent SEM study has provided comprehensive data for understanding three-dimensional structures of the adrenal cortex, especially the parenchymal cell arrangement and the perisinusoidal space for nutrient exchange between the blood plasma and the parenchymal cell.

Furthermore, cytological examinations have revealed the presence of, besides macrophages, perisinusoidal cells (Zelandier, 1964) which may be categorized as fibroblastic cells (Yamamoto et al., 1978), or interstitial cells belonging to the vitamin-A storing cell system (Hiroswa and Yamada, 1975; Yamada and Hiroswa, 1976; Hiroswa, 1977; Yamada, Hiroswa and Yorifuji, 1981).

The present study aims to describe the surface fine structure of the perisinusoidal cells in a three-dimensional organization of the monkey adrenal cortex, whose cytological structure is known to be almost the same as that in other species of mammals (Brenner, 1966; Penny and Brown, 1971).

MATERIALS AND METHODS

Adrenal glands of adult macaque monkeys weighing about 2 kg. These monkeys were anesthetized with an intraperitoneal injection of pentobarbital (25 mg/kg) and perfused through the abdominal aorta with 100 ml of warmed Ringer's solution, and then with 2.5% glutaraldehyde in 0.1 M Sörensen's phosphate buffer (pH 7.4) at room temperature. In two monkeys, chicken erythrocytes suspended in Ringer's solution were injected into the femoral artery about 30 min prior to perfusion. The blood cell suspension was prepared as follows: blood from decapitated chickens was heparinized and centrifuged; the blood cells thus collected were fixed by dispersion in 1% formaldehyde for 1 day; and fixed blood cells were washed for a week by three changes of Ringer's solution a day under constant agitation to remove excess aldehyde, then re-suspended in Ringer's solution. After perfusion the adrenal glands were excised from the animals and immersed for a week in the above fixative of glutaraldehyde.

Aldehyde-fixed adrenals were fractured by hand to expose the surfaces for SEM observation, cut into small blocks (about 5 x 5 x 3 mm), and immersed in 2% tannic acid for 2 hrs. After rinsing with Sörensen's buffer (pH 7.4) overnight, the specimen blocks were postfixed with 1.33% osmium tetroxide, dehydrated in a series of graded alcohol and dried by a critical point method of CO2. Fractured surfaces for observation were coated with gold in an ion coator and examined under a Hitachi S-450 type SEM.

For the TEM observation, small blocks of glutaraldehyde-fixed tissues were cut into small pieces and postfixed with 1.33% osmium tetroxide in 0.1 M Sörensen's phosphate buffer (pH 7.4) for 2 hrs. Postfixed specimens were dehydrated with a series of graded alcohol, transferred into propylene oxide, and embedded in Epoxy resin. Thin sections were cut from plastic embedded specimens and stained with uranyl acetate and lead nitrate. Observation of the thin sections was performed in a Hitachi H-500 TEM.

RESULTS

On crosswise fractured surfaces of the adrenal cortex in the monkey, three zones corresponding to the histological zonation were clearly distinguished (Fig. 1): the zona glomerulosa in globular lumps and circular hollows (Fig. 2); the zona fasciculata; and
the zona reticularis in palisade or latticed extensions of laminae (Fig. 1). These lumps and laminae were formed by close apposition of polyhedral parenchymal cells and were separated by intervening sinusoids.

The circular hollows in the zona glomerulosa were cut-open surfaces of the cell lumps (Fig. 2). Cuboidal cells were closely packed on their lateral surface, forming acini. The cell surface, especially the lumenal surface of the acini, was hairy with numerous microvilli. The microvilli were finger-like and about 0.5 μm long. On the dissociated lateral surfaces of the parenchymal cells in all zones were always seen smooth areas distinct from the surrounding hairy areas (Fig. 2, 3b). The areas were sometimes equipped with a centrally located depression or protrusion (Fig. 3b) which was encircled with a concentrical arrangement of many small processes. The areas were presumed to represent the dissociated attachment between adjoining parenchymal cells. Intercellular fibrils were not seen in the areas.

The sinusoids appeared as widened furrows or hollows and varied in diameter from about 7 to 15 μm wide, being lined with only a continuous layer of flattened endothelial cells (Fig. 3a, 4a). The perikarya usually bulged into the sinusoid lumen. The
Fig. 3. Fractured surface of the zona fasciculata. Cuboidal parenchymal cells (P) are closely stacked in laminae along the sinusoid (S). a. Subendothelial surfaces of the cell laminae are seen by partial removal of the endothelial lining. Numerous microvilli on the parenchymal cells are entangled with collagen fibrils (CF). x 2,400 b. Dissociated lateral surfaces of parenchymal cells in a cell lamina. Relatively smooth areas are distinct from surrounding rough areas and equipped with depression (black arrow) or swelling (white arrow). The endothelium lining the sinusoid (S) shows a perikaryal swelling (upper left corner). CF collagen fibrils in perisinusoidal space. x 5,100
The lumenal surface of the cells was relatively smooth, except for some globular or short microvilli. Furthermore, fenestrations (about 0.1 μm in diameter) on the thin endothelial cytoplasm were either disseminated, or arranged in groups, forming sieve plates (Fig. 4b, 5). Large endothelial pores (Motta, Muto and Fujita, 1979) were not observed in any zone of the cortex in this species. Endothelial perikarya frequently showed prominent protrusions, from which thick cytoplasmic crests extended and were transferred into the peripheral cytoplasmic layer. The perikarya were sometimes equipped with groups of disc-like processes (Fig. 4b). Twenty or more processes were often counted in a group. These processes were fan-like discs in various diameters which ranged from 0.5 μm to 1 μm. The thickness was about 0.1 μm, almost corresponding to that of the fenestrated endothelial cytoplasm. The cytoplasmic discs were also perforated with numerous fenestrations with thin diaphragms (Fig. 5). The structural meaning of the processes is not known at present, though this type of processes has been previ-
viously reported in the glomerular capillaries and regarded as a precursory structure for partial renewal of the sieve plate of the endothelial cytoplasm (Wolff, 1966; Wolff and Merker, 1966; Fujita et al., 1976; Yoshinari and Fujita, 1982).

The cell boundary was noted as irregularly scalloped margins of overlapping peripheral cytoplasms or roughly protruded lines with marginal folds. Attachment structures were also developed between adjoining endothelial cells (Fig. 5).

Perisinusoidal spaces were always recognized as continuous, slit-like lacunae of various widths (about 0.3 μm) between the endothelial and parenchymal cells. The space contained a varying amount of fine fibrils of collagen (Fig. 3a, b, 6). The fibrils were in a diffused distribution, though sometimes in fascicles. These fibrils were entangled with numerous microvilli arising from parenchymal cells, and also extended into the intercellular spaces among the parenchymal cells in the zona fasciculata and reticularis (Pauly, 1957).

Irregularly shaped perisinusoidal cells were found in expanded perisinusoidal
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spaces or intercellular spaces among parenchymal cells. Two types of cells were distinguished from the viewpoint of the surface structure: stellate interstitial cells (Fig. 6) and monstrous wandering cells (Fig. 7a, b).

The interstitial cells were characterized by thick processes which emerged from the polygonal perikaryon and extended into variously formed branches among parenchymal cells (Fig. 5, 6). Marginal portions of the processes were usually in severe attenuations or spiny folds. The extension of the processes of an interstitial cell sometimes occupied the whole thickness of a cell lamina (Fig. 5, 6). The free surface of the cells was usually pitted with some depressions caused by the close adjoining of parenchymal cells. The processes were always entangled with collagen fibrils. In the TEM graphs of the interstitial cell (Fig. 5), the nucleus with condensed chromatin was invested with thin cytoplasm, in which cell organelles, such as Golgi apparatus, small cisterns of endoplasmic reticulum studded with ribosomes, and free ribosomes were well developed. One or two lipid droplets were also recognized.

The wandering cells were monstrous in their appearance. The perikaryon in a spherical (Fig. 11a), spindle-shaped (Fig. 7a) or stellate mass (Fig. 7b) was usually associated with cytoplasmic processes in various sizes. The processes were cylindrical or varicose threads with terminal branches or swellings. Furthermore, the processes extended in bipolar or radial directions. The surface of the cell body was usually
rough with the presence of tiny globular, or finger-like processes or blebs. The wandering cells were seen in the perisinusoidal spaces together with collagen fibrils (Fig. 11a), but also in the acinar lumens in the zona glomerulosa (Fig. 7a, b).

The TEM views of wandering cells showed a large, electron lucent nucleus invested in an ample amount of cytoplasm, which contained many spherical granules with dense, homogenous contents (Fig. 8, 11a). Sometimes, many residual bodies of phagosomes and one or two membrane-bounded lipid droplets were also seen.

Fig. 7. Wandering cells (M) in acinar lumens of the zona glomerulosa. a. Cell body in a spindle shape shows a smooth surface, except for tiny projections. Bipolar processes extend to the perisinusoidal space through intercellular spaces. ×2,800. b. This cell issues several processes in various sizes and forms. The cell surface is rough with many processes or swellings. P parenchymal cells, S sinusoid. ×3,200
In the monkeys intravenously injected with fixed chicken blood cells, many macrophages with phagocytized chicken erythrocytes were observed on the endothelial lining of sinusoids (Fig. 9, 10). These phagocytized erythrocytes were enveloped with thin, laminar processes of the macrophages. In the TEM images of the macrophages (Fig. 10), the nuclei were electron lucent, were surrounded by a large amount of cytoplasm, contained many dense granules about 0.5 μm in diameter, which were disseminated throughout the cytoplasm. The fine structural organization of the sinusoid macrophages was similar to that of the wandering cells in the perisinusoidal cells.

**DISCUSSION**

As described in Motta, Muto and Fujita's (1979) observations in the rat, cat and pig, the adrenal cortex in the monkey is also composed of interconnected groups in laminae of parenchymal cells. In the monkey, however, the grouped cells in the zona glomerulosa frequently formed acini with lumens, being bordered with numerous microvilli from the parenchymal cells. In the lumens were usually seen wandering cells, but not connective tissue elements such as interstitial cells nor fibrils of collagen. Therefore, the space is apparently different in its origin from the intercellular or perisinusoidal spaces.
Fig. 9. Macrophages (M) in a sinusoid. Their flap-like processes (arrows) extend on chicken erythrocytes (R) trapped by the cells. × 8,400

Fig. 10. TEM view of a macrophage (M) on the endothelial lining (E). Note the fine chromatin in a slightly indented nucleus. Abundant cytoplasm contains numerous small granules of lysosomal nature. Chicken erythrocytes (R) are embraced with the cytoplasm of the macrophage. P parenchymal cells. × 6,500
Two types of perisinusoidal cells which have been identified in the present study are considered to differ in nature from each other.

The stellate interstitial cells are assumed to be connective tissue cells, because they are always situated in the perisinusoidal space, and the shallow depressions on their perikarya and their spiny or attenuated processes might be due to the irregular forms of the perisinusoidal and intercellular spaces in which the cells are housed. Their surface morphology is identical with the SEM figures of interstitial cells or Ito’s fat storing cells in the liver (Ogawa and Miyoshi, 1976; Muto, Nishi and Fujita, 1977; Wisse, 1977; Nopanitaya, 1979). Furthermore, the cytoplasmic structure of the interstitial cells is the same as that of fibroblasts in the fibrous capsule (Brenner, 1966). The existence of lipid droplets suggests that the cells belong to the vitamin-A storing cell system (Yamada and Hirosawa, 1976; Hirosawa, 1977; Yamada, Hirosawa and Yorifuji, 1981) in the adrenal cortex, as the Ito cells do in the liver (Wake, 1980; Tanuma, Ito and Shibasaki, 1982).

The wandering cells in the intercellular space appear monstrous in surface structure due to pseudopodia-like processes. These processes may indicate that these cells are in the moving stage, while the spherical, hairy cells are in the resting stage. This interpretation of the surface structure in the wandering cells well coincides with that in macrophages in various organs (Miyoshi and Fujita, 1971; Carr, 1973; Hosoya and Fujita, 1973; Munthe-Kaas, 1976; Kessel and Kardon, 1979).

Although macrophages in the adrenal cortex are relatively few in number (Stuart, 1970), Motta, Muto and Fujita’s (1979) SEM observation in the pig has reported cytoplasmic processes of macrophages in pores of endothelial lining, which the present study in the intact monkeys has failed to find. However, the intravenous injection of chicken blood cells in the present study proved the presence of cells which were capable of phagocytizing foreign blood cells. The SEM images of the phagocytosis of the cells are essentially identical with those of Kupffer cells (Munthe-Kaas et al., 1976;
Muto and Fujita, 1977). Furthermore, the TEM observations that the macrophage cytoplasm contains an oval nucleus, well developed Golgi apparatus, and many small lysosomal granules support the theory that the cells are macrophages, showing the cell nature of the monocyte. The resemblance of the cytoplasmic structure between the macrophages on the hepatic sinusoidal wall and the wandering cells in the adrenal perisinusoidal space suggests that the wandering cells might be derived from hematogenus macrophages, possibly monocytes. This view is supported by the fact that no hematopoietic foci have been observed in the perisinusoidal space, though the possible occurrence of tissue macrophages has been proposed in some organs (Daems, Koerten and Soranzo, 1976; van Furth, 1980).

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