Brief Notes

Electron Microscope Observations on the Endoplasmic Reticulum in the Human Fetal Adrenal.*

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The human fetal adrenal is proportionally 10 to 20 times larger than the adult gland. Its large size is attributable to a histologically distinct central reticular zone known as the fetal zone. This zone is prominent throughout fetal life, but undergoes rapid involution in the first postnatal week (1). In the course of a study of the human fetal adrenal, electron micrographs were obtained of cells in the fetal zone. These cells are distinguished by having a very extensive, densely packed system of tubules which occupies most of the cytoplasmic volume and are considered to be morphologically homologous to the endoplasmic reticulum found in other cells. In addition to its tubular structure, and dense packing, the endoplasmic reticulum of the fetal zone cells differs from that of other cell types described by Porter (2) and Palade (3) in being predominantly free of attached particles. It is felt that the unique character of the endoplasmic reticulum in the fetal zone justifies a brief description of our findings.

The adrenal specimens were obtained in Stockholm, from human fetuses between 6 and 17 weeks of gestation. The fetuses were delivered either by Caesarian section or vaginal hysterotomy. One adrenal gland was dissected free of the surrounding tissue and placed in a pool of cold OsO_4. In the case of the Caesarian sections the time lapse between cessation of blood flow from placenta to fetus until the tissue was placed in OsO_4 was approximately 3 to 4 minutes. Fetuses delivered by vaginal hysterotomy necessitated a longer time lapse due to the procedure involved. However the degree of postmortal change between the two procedures appeared negligible. Small blocks of tissue, approximately 1 mm. cubed, were then cut from the cortex, and placed in a vial of fresh OsO_4 for 1 to 2 hours. The OsO_4 solution during this time was maintained at 4°C., in an attempt to minimize extraction of the cytoplasm as well as the effects of incipient autolysis (4). The OsO_4 was a 1 per cent solution buffered with veronal-acetate to a pH of 7.6 to 7.8 according to the method of Palade (5). The addition of sucrose to the fixative to increase the osmolar concentration, appeared to aid in preservation of the tissue. After fixation, the tissue was dehydrated in a series of graded acetone solutions, and then impregnated with a mixture of 9 parts butyl methacrylate to 1 part methyl methacrylate. A prepolymerized mixture of the same resin as used for the impregnation was utilized for the final embedding (6). Final polymerization of the methacrylate was carried out at 60°C. Thin sections were cut in the usual way and examined in an RCA model EMU-2E electron microscope. For purposes of orientation, thick sections were also cut, and observed with a phase-contrast microscope.

Fig. 1 shows a portion of a cell from the fetal zone. The cytoplasm at this low magnification appears to have a fine, dense granular texture. However, at higher magnification, as in Fig. 2, it may be seen that this "granular" element is actually a collection of small tubular structures. These structures have an outer limiting membrane and a homogeneous content. There is no particular orientation to these tubular structures. In fact, the membranous system comprising this agranular or smooth endoplasmic reticulum appears as a vast complex of tortuous tubules of slightly irregular diameter. The diameter of the tubules ranges between 250 and 300 A. Cells near the surface of a block of tissue frequently show ruptures of their limiting membranes, perhaps due to mechanical stress in the process of cutting the tissue immediately preceding fixation. With rupture of

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the cell membrane, there is a resultant dispersal of the cellular contents. The reticulum becomes dispersed enough so that its morphological nature can be clearly seen (Figs. 3 to 5). As far as the size and shape of the tubules is concerned, there is no notable difference between intact cells and cells with ruptured membranes. The tubules are only more tightly packed in the former.

At the high magnification of Fig. 3 an anastomosis or communication between tubules of the agranular or smooth endoplasmic reticulum is seen. Many of the profiles depart from the relatively uniform tubular shape in that they possess numerous constrictions along their lengths, resulting in a moniliform or bead-like appearance. Fig. 4 shows some of these constrictions clearly. In Fig. 5, a tangential section of the granular part of the endoplasmic reticulum is shown. At both ends of the profile there is continuity with the agranular reticulum. This continuity between the two profile varieties of the endoplasmic reticulum has previously been demonstrated in liver cells (7). The precise mode of communication between the tube-like structures of the agranular reticulum and the cisternal structures of the granular reticulum cannot be clearly seen here. However, there is an indication that the agranular reticulum balloons or expands when it is in proximity with the granular reticulum, resulting in a continuous membranous system.

Small dense granules approximately 150 A in diameter are confined to some of the flattened vesicles of the endoplasmic reticulum, giving it a "rough" appearance. These granules are assumed to be the equivalent of those found in secretory cells, and thus composed of ribonucleoprotein (RNP). However, positive identification should await chemical analysis as has been done in the case of liver and pancreas (7, 8). We have seen no granules distributed in the cytoplasm free of a membrane.

The occurrence of masses of tightly meshed, smooth surface endoplasmic reticulum have been observed before, but they were never developed to the extent encountered in the fetal zone cells. Porter (9) denotes this structure to be common in cells with a shape to maintain as in the sustentacular cells of the olfactory epithelium and in crown cells found in the saccus vasculosus. Fawcett (10) has found a conspicuous compact network of small vesicles and short tubules in the liver cells of animals recovering from a period of fasting which he believes to represent an early stage in the regeneration of the granular endoplasmic reticulum. Similarly Palade and Siekevitz (7) describe an agranular reticulum in normal rat liver occurring in irregular agglomerations usually located towards the periphery of the cell. The recurrence of this reticulum has been demonstrated in many other cell types (8). In consideration of function it is of interest to note that according to Palade (3), secretory cells engaged in active protein synthesis are made up primarily of elements of the rough surface endoplasmic reticulum containing RNP granules, whereas the smooth surface membrane system, with no attached granules, is found to predominate in cells active in lipide metabolism. This observation correlates with the high steroid biogenetic capacity of fetal zone cells which has been observed in previous studies (11). The question as to the precise function of the fetal zone still remains unresolved. However, it seems likely that further inquiry in regard to the nature and activity of the agranular endoplasmic reticulum may prove advantageous in understanding the role of these specialized fetal cells.

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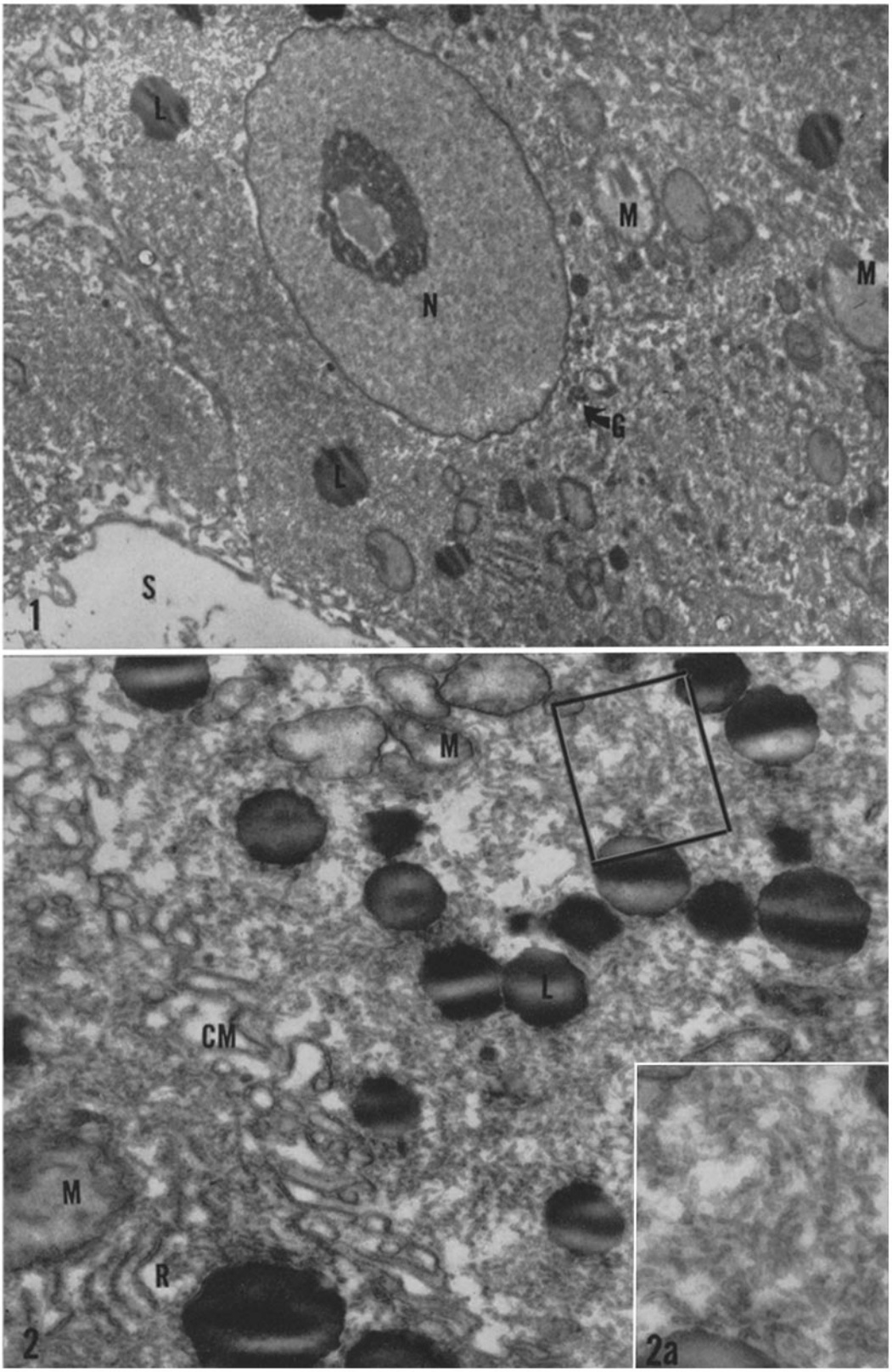
Explanation of Plates

Plate 325

Fig. 1. Electron micrograph, low power, of a portion of a cell of the fetal zone. At this magnification the cytoplasm has a fine granular appearance especially in the region below and to the the left of the nucleus (*N*). Numerous mitochondria (*M*) and granules (*G*) both of varying sizes are located in the right half of the micrograph. The very dense structures (*L*) are lipide droplets. A blood sinusoid (*S*) separated by a very thin and somewhat poorly defined endothelium is shown in the lower left of the figure. × 8,000.

Fig. 2. A vast collection of small tubule-like structures, the agranular endoplasmic reticulum, is seen at a higher magnification in a cell similar to that shown in Fig. 1. The tubules that form the reticulum are disposed at random as indicated by the proportion of circular, oval, and elongated profiles. The round dense structures are lipide droplets (*L*) and a number of mitochondria (*M*) can be seen. The cell membranes (*CM*) of two adjacent cells are seen to interdigitate forming a complex array of the membranes. A small area containing endoplasmic reticulum associated with granules (*R*) is also evident. × 22,000.

Fig. 2 A. The inset shows at a higher magnification the area outlined in Fig. 2 for comparison with Figs. 3 to 5. × 39,500.



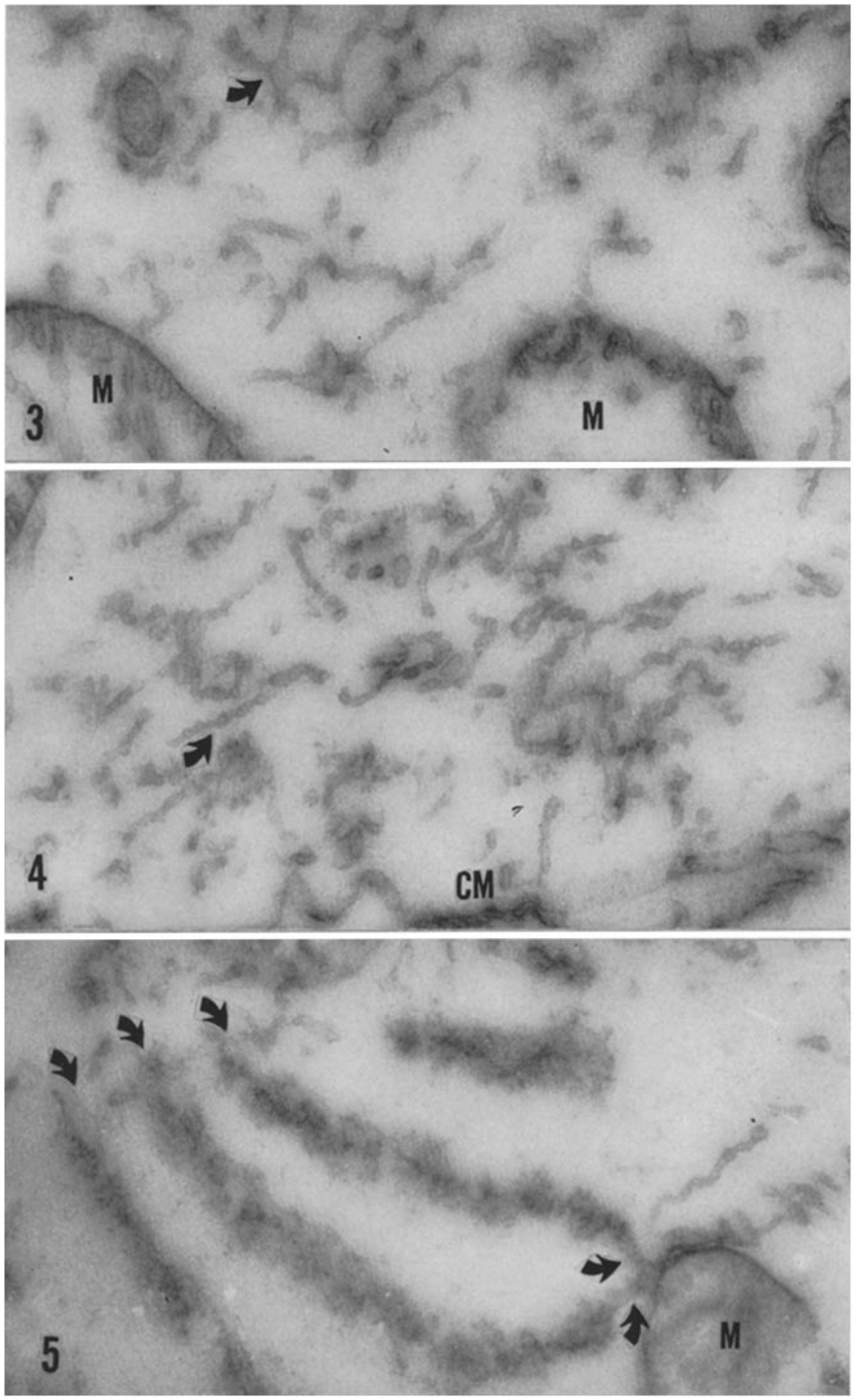
(Ross *et al.*: Endoplasmic reticulum in fetal adrenal)

PLATE 326

FIG. 3. A high magnification micrograph of the interior of a cell whose outer limiting membrane has ruptured. Some of the cytoplasmic contents of the cell have spilled out leaving the remaining cytoplasmic structures relatively dispersed. Because of this dispersion the morphological features of the endoplasmic reticulum can be seen to greater advantage in this and the following two electron micrographs. The arrow here shows an anastomosis or communication between several of the tubules. The mitochondria (*M*) have tubular internal membranes, the dimensions of which are similar to those of the tubules of the agranular endoplasmic reticulum. × 40,000.

FIG. 4. High power of the agranular endoplasmic reticulum similar to Fig. 3. The arrow indicates one of the profiles that shows numerous constrictions along its length producing a moniliform appearance. At the lower border of the micrograph two adjacent cell membranes (*CM*) are seen. × 40,000.

FIG. 5. A tangential section through an array of lamellae of the granular ("rough") endoplasmic reticulum. The arrows at both ends of the profiles indicate the continuity existing between the membranes of the granular and agranular components of the endoplasmic reticulum. A portion of a mitochondrion (*M*) is seen adjacent to the reticulum. × 40,000.



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