PEROXISOMES IN INNER ADRENOCORTICAL CELLS
OF FETAL AND ADULT GUINEA PIGS

VIRGINIA H. BLACK and BRUCE I. BOGART

From the Department of Cell Biology, New York University School of
Medicine, New York 10016

ABSTRACT

Abundant membrane-bounded granules, 0.1–0.45 μm in diameter, occur among the elements of the smooth-surfaced endoplasmic reticulum in zona fasciculata and zona reticularis adrenocortical cells of guinea pigs. Acid phosphatase cannot be cytochemically demonstrated in them, and they are therefore distinct from lysosomes. Incubation in medium containing 3,3'-diaminobenzidine results in dense staining of the granules, identifying them as peroxisomes. These small peroxisomes increase in number as fetal adrenocortical cells differentiate, and they appear to arise from dilated regions of endoplasmic reticulum. They maintain interconnections with the smooth endoplasmic reticulum and with one another.

Peroxisomes or microbodies are granules which are bounded by a single membrane and have a homogeneous particulate content. They were first described by Rhodin in proximal convoluted tubule cells of the kidney (71). Soon after, Gänsler and Rouiller (31) identified them in liver hepatocytes. They have now been found in a wide variety of cell types, both plant and animal (see reviews 38, 39). Biochemically they are characterized by oxidative enzymes which generate H_2O_2, such as uricase and D-amino acid oxidase, and by catalase, an enzyme which breaks down H_2O_2 (5, 22). Cytochemically they can be identified by their staining with 3,3'-diaminobenzidine (DAB) (60).

In the proximal tubule cells of kidney (7, 71) and in liver hepatocytes (31), the peroxisomes are about 0.5 μm in diameter. Those in liver contain a central nucleoid, believed to represent highly concentrated uricase (5, 40, 77, 78). Within the last few years, however, smaller peroxisomes, about 0.2 μm in diameter, have been found in a wide variety of tissues (2, 6, 20, 27, 28, 41, 45, 54, 62, 65, 69). These generally lack a central nucleoid and, on the basis of morphology alone, are easily confused with primary lysosomes. However,

cytochemical studies have defined them as a separate organelle and allowed their classification as peroxisomes (6, 20, 62, 69). Recently, Novikoff and Novikoff (62) emphasized that such small peroxisomes are extensively interconnected with the endoplasmic reticulum and suggested that this may be true of all small peroxisomes, or microperoxisomes, as they prefer to call them.

Small peroxisomes have been described in steroid-secreting cells of adult animals (6, 41, 54, 69), but clear continuities with endoplasmic reticulum have not been demonstrated. This paper confirms earlier reports (13, 41) that the small granules in the adrenocortidal cells of guinea pigs are peroxisomes and clearly demonstrates their close interrelationship with the endoplasmic reticulum. In addition it describes the striking increase in numbers of these organelles which occurs during differentiation and emphasizes their possible significance in steroid-secreting cells.

MATERIALS AND METHODS

English short-haired guinea pigs (Camm Research Institute, Inc., Wayne, N. J.) used in this study were

bred according to procedures previously described (12, 14).

Morphology

Adrenals from fetuses 19–65 days of gestation and from five adult animals were fixed as 1 mm³ pieces for 4–6 h in 3% glutaraldehyde buffered to about pH 7.4 with 0.2 M sodium phosphate or 2% Dalton's dichromate buffer. They were rinsed in these same buffers and fixed the following day in phosphate-buffered osmium tetroxide (57), stained in block with 0.5 or 1% aqueous uranyl acetate, and embedded in Epon. Sections showing pale gold or silver interference colors were cut on a LKB III ultratome (LKB Instruments, Inc., Rockville, Md.), stained with lead citrate (79) or with both uranyl acetate and lead citrate, and examined on a Siemens Elmiskop IA.

Cytochemistry

Adrenals taken from fetuses 26–55 days of gestation and from adult males and females were cut into 1 mm³ pieces and fixed for 4–6 h in 3% glutaraldehyde buffered to pH 7.4 with 0.2 M sodium cacodylate (72). They were rinsed for at least 12 h in 0.1 M sodium cacodylate buffer before chopping into 25–50 μm thick sections on a Sorvall TC-2 tissue sectioner (Ivan Sorvall, Inc., Newtown, Conn.).

Some sections were incubated in Gomori's medium as modified by Barka and Anderson (4), for the cytochemical demonstration of acid phosphatase activity. Control sections were incubated in medium without substrate. Sections were rinsed three times in acetate-Veronal buffer (74) and postfixed in osmium tetroxide buffered with acetate-Veronal (64).

For cytochemical identification of peroxisomes, tissue sections were incubated in DAB oxidation medium at pH 9.0 according to the procedures of Novikoff and Goldfischer (60) as modified from Graham and Karnovsky (34). Control experiments consisted of adding 0.1% catalase or 0.2 M aminotriazole to both the incubation and preincubation media. Some sections were incubated in medium lacking H_2O_2. Other sections were placed in a water bath at 80°C for 20 min before incubation, in an attempt to heat-inactivate the enzymes of the peroxisomes.

After incubation, sections were rinsed in 0.1 M sodium cacodylate buffer and fixed in cacodylate-buffered osmium tetroxide. Osmium-fixed sections were dehydrated and embedded in Epon. Some sections were stained in block with 0.5 or 1% aqueous uranyl acetate before dehydration. Thin sections, showing pale gold or silver interference colors, were taken with a LKB III ultratome. Some thicker sections, showing dark gold, brown, or purple interference colors, were also taken. Sections were examined with a Siemens Elmiskop IA electron microscope either unstained or after staining with uranyl acetate or lead citrate (79) or with uranyl acetate followed by lead citrate.

OBSERVATIONS

The histogenesis and fine structure of fetal guinea pig adrenals has been described by one of us in detail elsewhere (12). It will be briefly reviewed here for orientation.

Fetal guinea pig adrenals become recognizable at 21–22 days, roughly one-third of the way through their 65–70 day gestation period. They appear as a cluster of polyhedral-shaped cells slightly anterior, as well as dorsal and medial, to the genital ridge. By 24–26 days the cells increaes in number forming a grossly recognizable adrenal

FIGURE 1   At 26 days, inner adrenal cortical cells are filled with loosely arranged tubular endoplasmic reticulum. Polyribosomes occur at widely spaced intervals on the surface of the tubules (short arrows) leaving the reticulum predominantly smooth surfaced. Small granules, with a uniform, coarsely particulate content, similar in appearance to that of peroxisomes (p), are scattered throughout the cytoplasm. Occasionally they appear to be in continuity with the endoplasmic reticulum (long arrows). Ribosomes (short arrows) are found a varying distance along the reticulum from these regions of apparent continuity. Other, lysosome-like granules (l) with a more electron-opaque fine particulate content are also seen in the cells. × 23,750.

FIGURE 2   By midterm, the smooth-surfaced endoplasmic reticulum becomes tightly packed in the cytoplasm of inner cortical cells. It consists of randomly interweaving tubules and organized arrays of parallel tubules. Ribosomes are confined to sparse patches on the reticulum (short arrow). The small membrane-bounded granules (p) are more numerous in the older cells, as shown in this cell from a 40-day old fetus. They are scattered among the randomly arranged tubules of the endoplasmic reticulum. They occur between arrays of parallel tubules, but not between the tightly packed tubules within the arrays. Occasionally two granules can be seen interconnected by a narrower element, similar in diameter to the tubular smooth reticulum (long arrow). × 31,000.
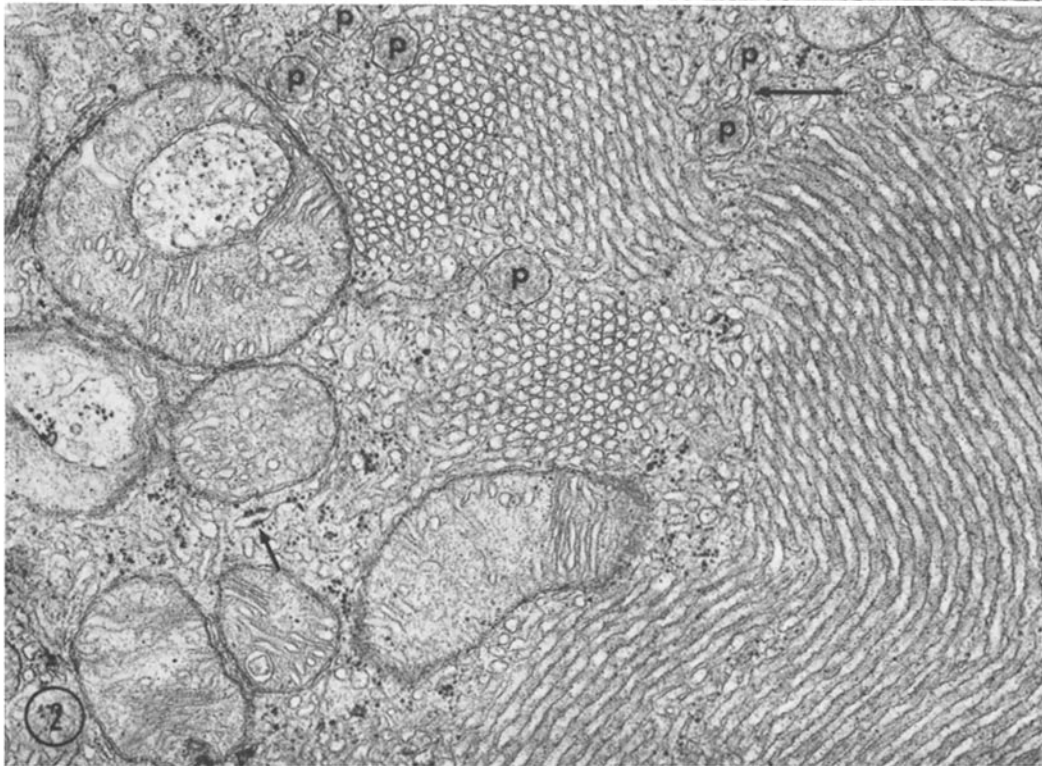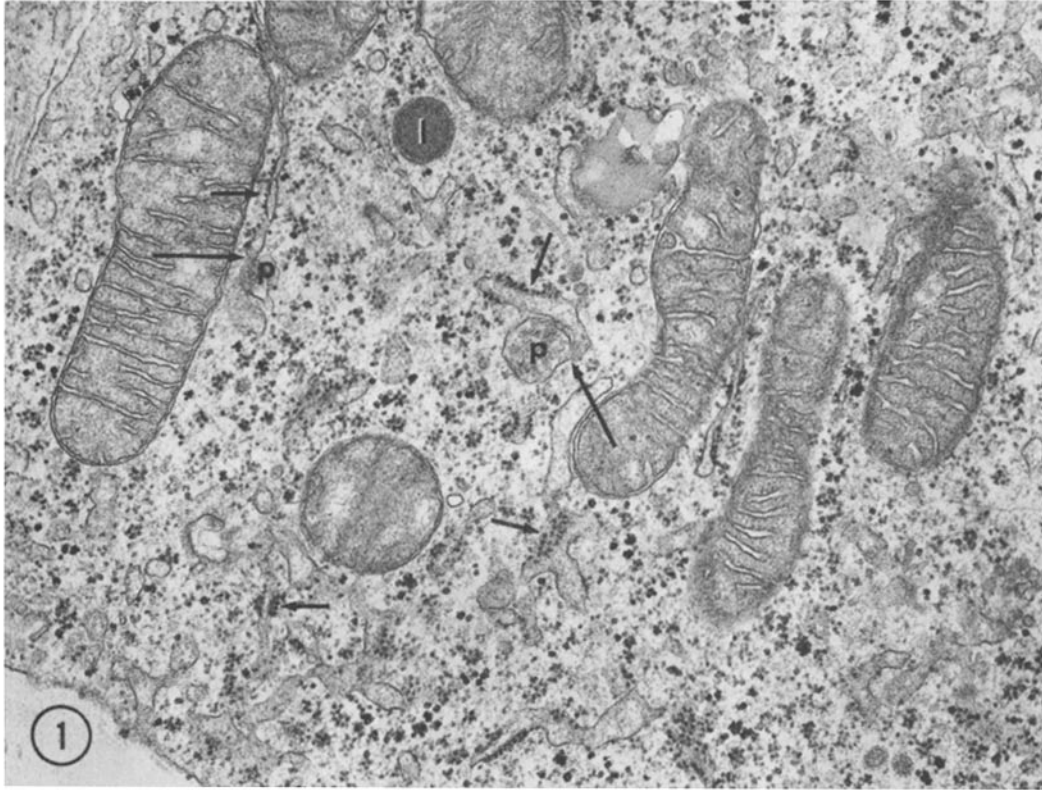
gland with two histologically distinct zones of cells. As the adultlike zonation pattern develops toward late term, the outer zone of small cells gradually forms the zona glomerulosa and the inner zone of large cells gradually becomes the zona fasciculata and zona reticularis.

Ribosomes, which are found in abundance on the endoplasmic reticulum of cortical cells at 21–22 days, become more sparsely distributed with age (Fig. 1). Eventually they are confined to short rough-surfaced cisternae and to a few patches of polyribosomes scattered on random tubules (Figs. 2, 3).

Smooth-surfaced endoplasmic reticulum first appears in the cortical blastema cells at 22 days. It increases in amount dramatically in the inner zone cells, filling the cells with loosely organized, predominately smooth-surfaced tubules by 26 days (Fig. 1). By midterm, 27–30 days, the inner cortical cells are packed with tightly interwoven, randomly arranged tubules (Figs. 2, 3). In some areas of the cytoplasm of these cells parallel tubules become organized into paracrystalline arrays (Fig. 2). Whorls of fenestrated smooth-surfaced cisternae also appear in the cells at this time. By late term, 55 days and older, cells containing such complex paracrystalline and whorled arrays become localized to the deep zona fasciculata and

the zona reticularis. They continue to be found in these regions in postnatal and adult animals.

Membrane-bounded bodies, 0.1–0.45 μm in diameter, containing a fairly homogeneous but somewhat coarsely particulate content, are scattered among the elements of endoplasmic reticulum in fetal and adult inner cortical cells (Figs. 1–7). Occasionally their membranes appear to be in continuity with those of the endoplasmic reticulum (Figs. 1, 3) and with membranes bounding other similar particulate-containing structures (Fig. 2). Most of these bodies are round or oval in shape and appear as granules scattered throughout the cytoplasm (Figs. 2, 3). A few are more elongated and appear similar to dilated regions of the endoplasmic reticulum (Fig. 4). Such dilated regions of reticulum may be constricted in one or more places along their length (Fig. 5).

Ribosomes are occasionally present on the surface of the dilated particulate-containing regions of the reticulum (Figs. 4, 6) and on the membranes bounding the rounded granules (Figs. 3, 7). They occur more frequently along the reticulum membranes at varying distances from regions of apparent continuity of these structures with the endoplasmic reticulum (Figs. 1, 6, 7). In sections the ribosomes appear as single ribosomes (Figs. 1, 3, 4, 6, 7). In surface view those on the reticulum appear as polyribosomes (Fig. 7).

FIGURE 3 In cells from older fetuses, continuity of the small granules (p) with the endoplasmic reticulum appears most frequently in regions where tubules of reticulum are randomly interwoven, as shown in this cell from a 40-day old fetus. In fortuitous sections these may be quite clear (long arrows). In other instances the images are merely suggestive (arrowheads). Occasionally ribosomes (r) are seen on the membrane bounding the granules. Ribosomes also occur on short segments of the tubular reticulum (short arrows). × 50,000.

FIGURE 4 Particulate material of a texture similar to that seen in the small granules occurs in elongated bodies which appear to be dilated lengths of the endoplasmic reticulum (arrows). Ribosomes (r) are occasionally seen on the membranes bounding these structures. Fetus, 45 days. × 35,000.

FIGURE 5 These dilated lengths of reticulum occasionally appear to be constricted (arrow). The more expanded portions are of a diameter similar to that of the individual small granules seen throughout the cytoplasm. Fetus, 40 days. × 54,000.

FIGURE 6 The more elongated membrane-bounded bodies interconnect with the tubular reticulum. Note the ribosomes (short arrows) on the surface of the membrane bounding the particulate content. Fetus, 27 days. × 39,000.

FIGURE 7 Individual small granules (p) are also seen interconnected with narrower diameter tubular elements of the reticulum (long arrows). Polyribosomes shown here in surface view (short arrows) occur on the surface of the tubules at variable distances from the interconnections. Single ribosomes (r) occasionally occur on the membrane bounding the granules. This is seen more frequently in young fetal cells where ribosomes are relatively more abundant on the reticulum surface and the tubules of reticulum are not so tightly interwoven. Fetus, 30 days. × 27,000.
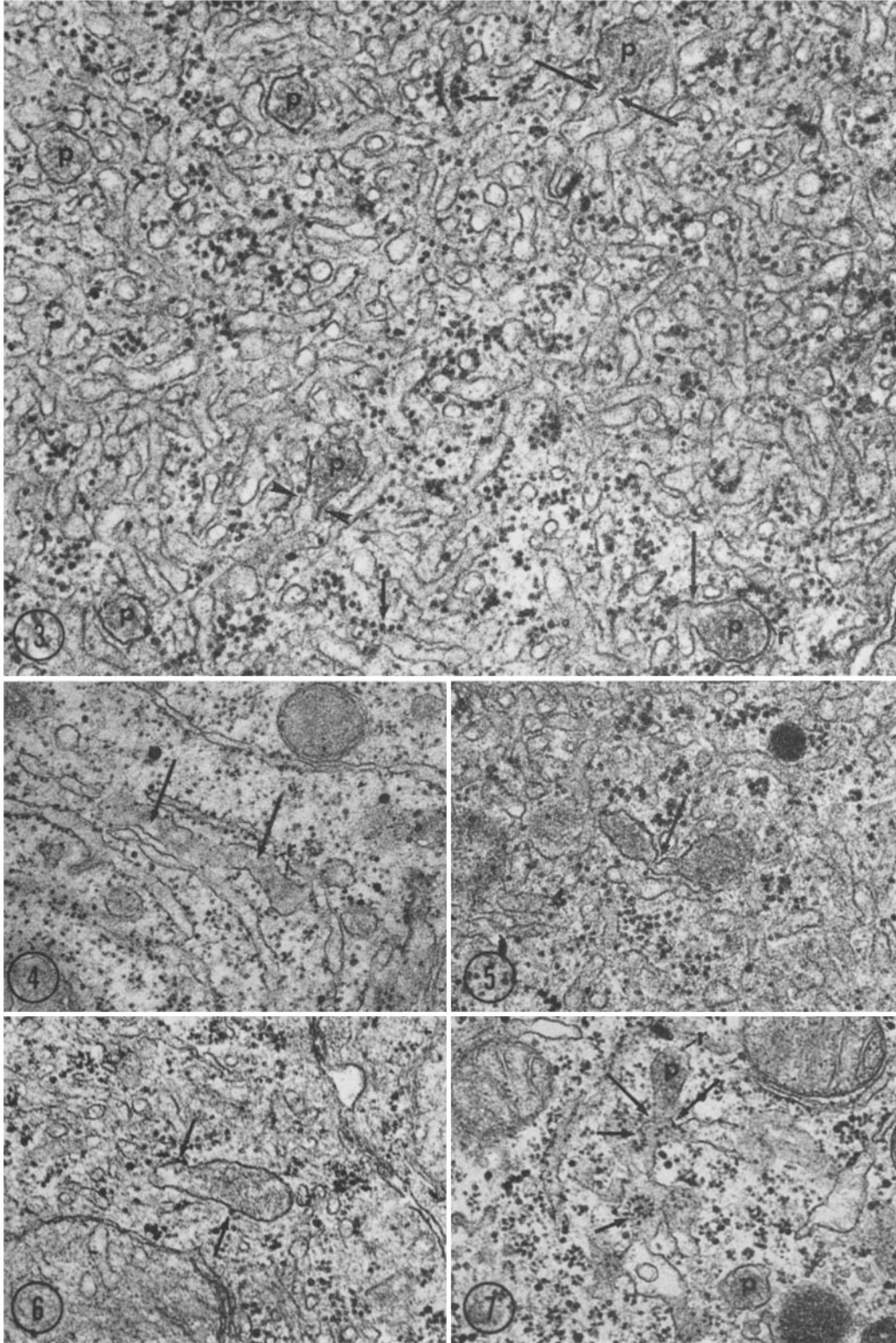
Ribosomes are seen most frequently, both on the membranes bounding the granules and on the adjoining reticulum, in young fetuses, 26–30 days old, when rough-surfaced reticulum is more abundant in the cells (Figs. 1, 6, 7). However, single ribosomes are occasionally seen in these locations in cells from older fetuses (Fig. 3).

These granular bodies seem to increase in number in the fetal cortical cells with age (Figs. 1–3), but precise quantitation is difficult due to the similarity between their electron density and that of the rest of the cell cytoplasm. In young cells the granules are randomly scattered throughout the cytoplasm. As the reticulum increases in amount and becomes arranged in complex arrays, the granular bodies become most abundant in areas of randomly arranged tubules (Fig. 3), and less abundant in areas of complex arrays of reticulum (Fig. 2). In older fetal and adult cells the granules are found between adjacent arrays of parallel tubules, but do not occur between the tightly packed tubules of any one array (Fig. 2). They are also less abundant in areas where whorled cisternae are tightly packed.

However, incidences of clear continuity of the small rounded granules with elements of the endoplasmic reticulum are less obvious in older fetal and adult cells. In cells from 22–26-day old fetuses elements of the endoplasmic reticulum are straighter and spaced farther apart (Figs. 1, 7). In these cells small granules can clearly be seen confluent with the reticulum, and the reticulum membranes can be visualized for some length. With age, as the reticulum becomes increasingly tubular it becomes more convoluted. Long elements of the reticulum become less frequently seen in section, and interconnections are less obvious (Figs. 2, 3). Close examination, however, reveals an increase in the number of granules showing continuity with short smooth-surfaced elements of the reticulum (Fig. 3). Such interconnections appear to be more frequent with randomly arranged tubules of endoplasmic reticulum than with parallel tubules in paracrystalline arrays or with whorled cisternae.

Other membrane-bounded granules within the cells which are larger, about 0.5 μm in diameter, have a more electron-opaque content of a finer particulate nature than that of the membrane-bounded bodies described above (Fig. 1). They appear similar to lysosomes, but are difficult to distinguish from the other bodies described above, except by the texture of their particulate content (Fig. 1). These granules also increase in number

with age, but become most numerous in the vicinity of the Golgi complex.

Cytochemical Results

After incubation in Gomori's acid phosphatase medium, reaction product was deposited upon the larger, more electron-opaque granules with finer particulate content (Figs. 8, 9). This identifies them cytochemically as lysosomes. No reaction product was deposited upon the smaller, less electron-opaque granules with somewhat coarser particulate content (Figs. 8, 9). These bodies, therefore, are not lysosomal in nature. Controls were devoid of reaction product.

After incubation in DAB oxidation medium the particulate contents of the smaller granules were stained intensely, identifying these bodies as peroxisomes (Figs. 10, 11). No staining was seen in lysosomes (Fig. 10). DAB also stained the particulate content of the more elongated membrane-bounded bodies which are similar in appearance to dilated lengths of endoplasmic reticulum (Figs. 12, 13). Stained particulate material was observed in the endoplasmic reticulum adjoining both the rounded and the more elongated particulate-containing bodies (Fig. 14). Slightly thicker sections gave an impression that this interconnecting system is quite extensive (Fig. 15). Ribosomes on the endoplasmic reticulum adjoining these structures did not stain. The intensity of staining in controls preincubated and incubated in medium containing catalase or aminotriazole was reduced. Absence of H_2O_2 from the medium did not appreciably affect stain intensity. Little or no staining occurred in heat-treated tissues incubated in medium containing aminotriazole.

Quantitation of peroxisomes was made much easier by the heightened density of the particulate content after DAB incubation (Fig. 11). Examination of the DAB-stained tissues confirmed, amplified, and clarified the observations made in material fixed for routine electron micrsocopy. Though no statistical analysis was done, the number of peroxisomes within the cells clearly increased with age. In cortical cells from adrenals of 22–26-day old fetuses one or two peroxisomes occur per cell section. By midterm (30–35 days) as many as 20 and by late term (55–65 days) as many as 50 peroxisomes are seen per cell section, scattered among the elements of the smooth-surfaced endoplasmic reticulum. The granules continued to be abundant in the adult cortical cells. Since no increase in the size of individual
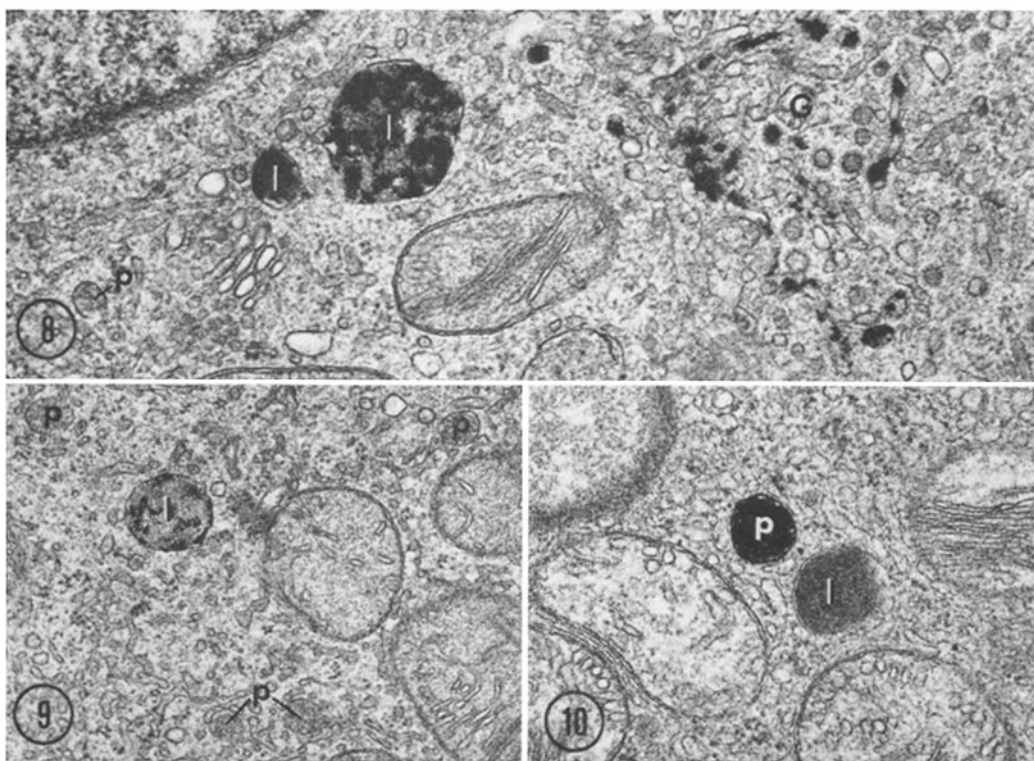
FIGURE 8   After incubation to demonstrate acid phosphatase activity, dense reaction product is seen in cisternae and vesicles on the concave face of the Golgi complex (G) and on the larger, more electron-opaque granule (l). Reaction product is not deposited on the smaller, less electron-opaque granules (p). Fetus, 52 days. × 28,000.

FIGURE 9   This micrograph depicts more clearly the deposition of reaction product, indicative of acid phosphatase activity, over the lysosomes (l) in comparison to the absence of reaction product over the small granules (p). Fetus, 53 days. × 25,000.

FIGURE 10   After incubation in DAB oxidation medium, the small granules (p) are stained, identifying them as peroxisomes. The lysosomes (l) are not stained, confirming that these are separate granule populations. Fetus, 42 days. × 32,000.

peroxisomes occurred with age, the increase in number of peroxisomes seen per cell section, coupled with the increase in cell size which occurs over this period (5 μm at 22 days, 8 μm at 30 days, 10 μm at 55 days), represents a great increase in the number of peroxisomes per inner adreno-cortical cell.

DISCUSSION

In animals, peroxisomes have been identified in kidney (7, 20, 71), liver (27, 28, 31, 49, 68, 77, 78, 82), small intestine (62), brown fat cells (2), adult steroid-secreting cells (6, 41, 54, 69), bronchiolar and aveolar epithelium (65), anal gland (45),

insect fat bodies (52), and the protozoan *Tetra-hymena* (58, 81). Peroxisomes described in proximal convoluted tubules of the kidney (7, 71), liver (31), anal gland (45), and insect fat bodies (52) are usually about 0.5 μm in diameter and contain a crystalline nucleoid. Those seen in the other tissues are smaller, 0.1–0.45 μm in diameter, and usually do not have a nucleoid. These smaller peroxisomes, because of their size and lack of a distinguishing nucleoid, are rather easily confused with primary lysosomes. However, they are cytochemically distinguishable from lysosomes; acid phosphatase cannot be demonstrated in them and they do stain with DAB (2, 6, 62, 65, 69).

The smaller membrane-bounded bodies seen in guinea pig adrenocortical cells are similar in appearance to the smaller peroxisomes described in several other tissues of adult animals (cited above). They stain with DAB, but contain no cytochemically demonstrable acid phosphatase activity. They are therefore peroxisomes and form a population distinct from lysosomes. DAB also stained the particulate content of endoplasmic reticulum adjoining some granules and that of elongated membrane-bounded bodies, which appear similar in form to dilated lengths of endoplasmic reticulum.

In several cell types it has been suggested that peroxisomes arise from the endoplasmic reticulum (27, 28, 52, 61, 62, 78), largely on the basis of their continuity with the reticulum. In addition, ribosomes have been seen on the membranes bounding the peroxisomes in rat fetal liver (78) and on the endoplasmic reticulum membranes close to and continuous with peroxisomal membranes in rat fetal liver (27, 28, 78) and insect fat bodies (52). In guinea pig adrenocortical cells many peroxisomes are in continuity with the endoplasmic reticulum. Ribosomes occur occasionally on membranes bounding the peroxisomes and more frequently on endoplasmic reticulum adjoining peroxisomes. They are also seen on the elongated particulate-containing structures which resemble dilated lengths of endoplasmic reticulum. The presence of the ribosomes confirms the endoplasmic reticulum origin of these membranes. The distribution of particulate material and ribosomes suggests that the particulate content of the peroxisomes

accumulates in regions of rough-surfaced endoplasmic reticulum, which lose their ribosomes, and that peroxisomes subsequently arise from these dilated regions.

There is some evidence that catalase is synthesized in precursor form within the endoplasmic reticulum and then sequestered into peroxisomes (48). One of these precursors is heme containing, the other is not (47). Porphyrin heme-containing compounds, such as catalase, bind partially oxidized DAB, and the staining of catalase can be distinguished from that of other porphyrin heme-containing compounds by its inhibition with aminotriazole (35–37). In this study DAB stained the particulate content of peroxisomes, of adjoining elements of endoplasmic reticulum, and of dilated lengths of endoplasmic reticulum. These results suggest that the particulate material seen in the apparent dilated regions of endoplasmic reticulum may represent the accumulation of catalase or its precursors in the endoplasmic reticulum before its sequestration into more distinct granules.

It has also been suggested that catalase is synthesized on ribosomes of rough-surfaced endoplasmic reticulum and enters the peroxisomes directly (49, 82). However, in this study staining of ribosomes did not occur, suggesting that this is not the mechanism of formation in these cells.

Interconnections between peroxisomes and endoplasmic reticulum, such as those described in this paper, have been reported previously (26, 52, 78). Recently, Novikoff and Novikoff (62) emphasized the frequency of such connections between

FIGURE 11 The DAB-incubated sections give a more accurate picture of the numbers of peroxisomes, as shown in this section from a 42-day old fetus. This section has not been counterstained with lead citrate. Some of the DAB-stained material is in elements with a diameter similar to that of the endoplasmic reticulum (arrows). × 17,900.

FIGURE 12 After DAB incubation the particulate contents of the dilated regions of endoplasmic reticulum are densely stained. A suggestion of staining in endoplasmic reticulum of narrower diameter is seen on the left. Fetus, 42 days. × 36,500.

FIGURE 13 The contents of constricted dilated regions of endoplasmic reticulum also stain intensely with DAB. Arrow denotes region of constriction. Fetus, 42 days. × 46,000.

FIGURE 14 The content of tubular reticulum adjoining such dilated regions also stains with DAB (arrow) × 46,000.

FIGURE 15 This micrograph is of a thicker section and gives some indication of the extensiveness of peroxisome-endoplasmic reticulum relationships. Two densely stained peroxisomes are shown interconnected by a narrow element (arrow). Densely stained material is also shown in tubular reticulum adjoining a peroxisome (arrow). Fetus, 42 days. × 32,000.
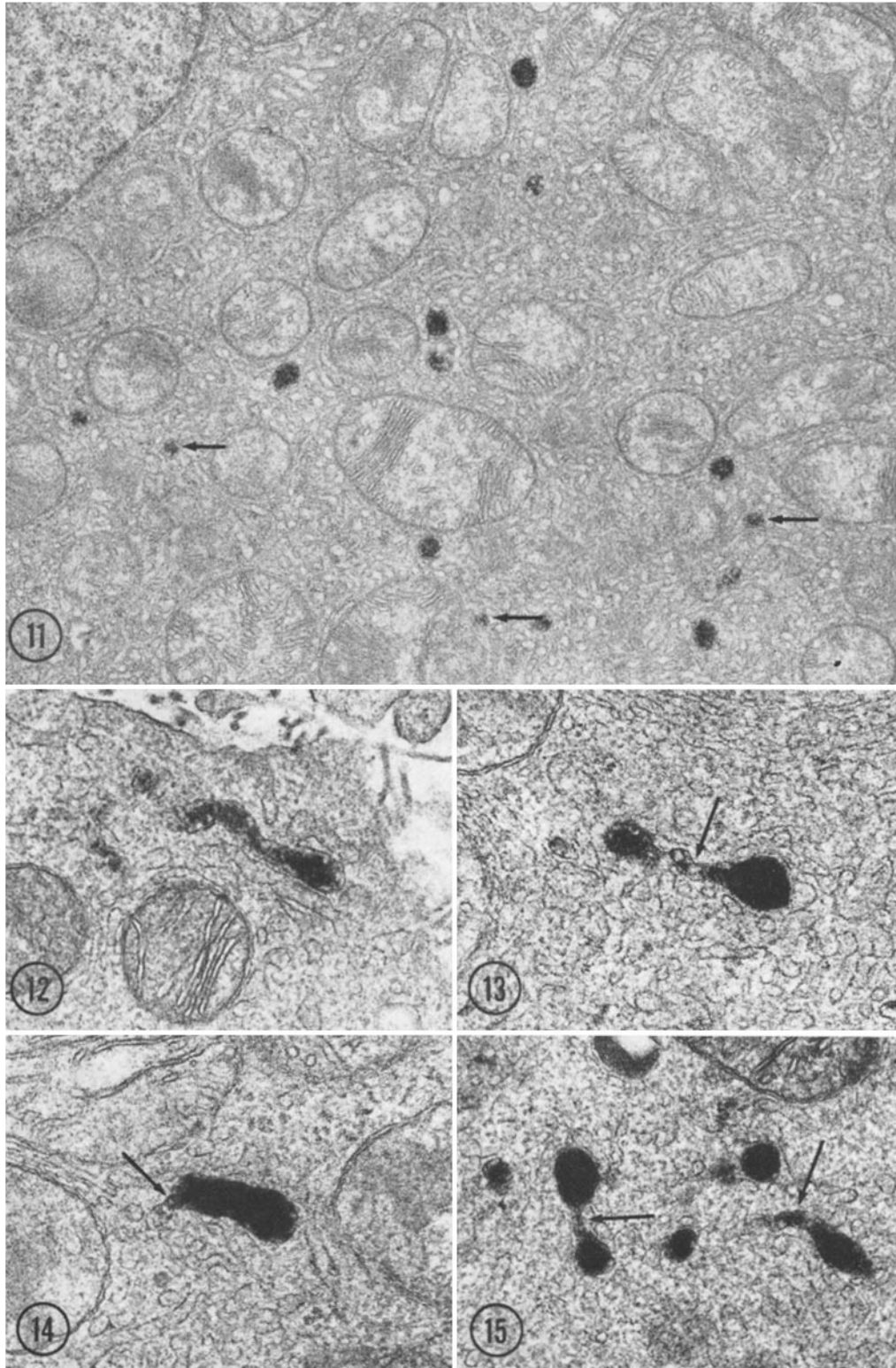
small peroxisomes and endoplasmic reticulum in intestinal epithelial cells. They called such small peroxisomes microperoxisomes, to emphasize their small size and to differentiate them from larger, nucleoid-containing peroxisomes. In fact, they considered the small peroxisomes or microperoxisomes to be just regional dilations of the endoplasmic reticulum. This would seem to imply that their position is static. The juncture between endoplasmic reticulum and peroxisomes is quite narrow. Whether the granules pinch-off or bud-off to form completely independent membrane-bounded bodies cannot be ascertained from a strictly morphological study. From our study we cannot say that all granules were interconnected with the endoplasmic reticulum. The frequency of interconnection between endoplasmic reticulum and peroxisomes, seen in random sections, suggests that such continuity is extensive. However, frequency does not imply constancy. It suggests only that continuity is either maintained or, if broken, quickly reestablished. The small peroxisomes in guinea pig adrenal cortical cells, like the microperoxisomes in intestinal epithelial cells (62), are in continuity with one another, as well as with the endoplasmic reticulum. This may suggest formation of multiple peroxisomes in one region, perhaps by constriction of dilated lengths of reticulum, or fusion of several peroxisomes formed in different regions of the cell cytoplasm. Whether such continuities are continuous or intermittent, the endoplasmic reticulum and small peroxisomes, microperoxisomes, form an extensively interconnected system, as recently emphasized by Novikoff and Novikoff (62). This may also be true for larger peroxisomes (68). Such interconnections may be of a biogenic as well as of a functional nature, a suggestion consistent with the biochemical data of Poole et al. (66, 67) which implies that constituents of peroxisomes such as catalase are freely exchanged between endoplasmic reticulum and peroxisomes.

Small granules, similar in appearance to peroxisomes, have appeared in the figures accompanying most articles describing steroid-secreting cells (1, 8–10, 12, 16–19, 21, 25, 29, 30, 32, 42, 43, 53, 56, 59, 70, 72). They were suggested to be peroxisomes or microbodies in a few reports (8, 12, 17–19, 28, 73), but more frequently went unnoted, were identified as secretory granules (9, 21, 70), or were confused with lysosomes (70). Very recent cytochemical studies (6, 41, 54, 69),

including this report, have confirmed their identification as peroxisomes.

The wide-spread occurrence of these organelles in steroid-secreting cells coupled with their striking numerical increase during differentiation, reported here, and their numerical changes with function (9, 16, 55) suggest that they play a significant, but as yet undefined, functional role in steroid-secreting cells. Some insight into their function, however speculative, might be gained by examining the possible relationship of known peroxisomal enzymes to steroid biosynthesis.

Peroxisomes have been associated with lipid metabolism in other cells (33). They are numerous in germinating seeds, where stored lipid is being converted to glucose (80), and are abundant in brown fat cells (2). Exogenously administered catalase, an enzyme found in peroxisomes, can decrease plasma cholesterol levels (3), and catalase may play a role in altering intracellular cholesterol levels (33). Steroid-secreting cells use cholesterol as the basic building material for steroid synthesis and there are several places along the steroid biosynthetic pathway where peroxisomal enzymes might participate in steroidogenesis.

The general pathway of steroid biosynthesis is well established and appears to follow a circuitous route through the cells (19, 24). While several enzymes involved in steroid biosynthesis are located in the mitochondria, may others are located in the microsomal fraction, which is believed to be derived largely from the smooth-surfaced endoplasmic reticulum (19). The close association of peroxisomes with smooth endoplasmic reticulum in steroid-secreting cells may, therefore, be of functional importance.

Peroxisomal enzymes may be involved in the formation of pyridine nucleotides necessary for the activity of the microsomal dehydrogenases and hydroxylases, involved in cholesterol and steroid hormone synthesis. The microsomal steroid dehydrogenases, Δ^5-3β and 17α-hydroxysteroid dehydrogenase, require NAD for their activity. The steroid hydroxylases, 17α- and 21-hydroxylase, located in the microsomal fraction, like those located in the mitochondria, require NADPH for their activity. de Duve and co-workers (22, 23) have suggested that enzymes in peroxisomes may act in concert with cell sap or microsomal dehydrogenases to reoxidize NADH to NAD. Peroxisomes may also function in generation of NADPH important for activity of the microsomal NADPH-

dependent hydroxylases. Glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase are potential generators of NADPH. Both enzymes have been identified in steroid-secreting cells. Although the microsomal glucose-6-phosphate dehydrogenase is emphasized in any consideration of generation of NADPH, peroxisomes may be involved in this function through isocitrate dehydrogenase which is reported, in liver cells, to constitute 25% of the protein in peroxisomes (50). If present in peroxisomes of steroid-secreting cells, it could provide an important generation system for NADPH.

Peroxisomes may be important in release of cholesterol stored in lipid droplets and cell membrane, as well as in its synthesis. Heme proteins such as catalase are powerful lipid peroxidation catalysts and may substitute for lipoxidases in animal tissues, where this enzyme is not found (75, 76). Boyd (15) has proposed that peroxidative formation of fatty acids may lead to rearrangement of cholesterol esters facilitating hydroxylations. Lipid peroxidation resulting in release of cholesterol from its esters would be important in steroid-secreting cells where cholesterol and cholesterol esters are stored in lipid droplets to be released upon pituitary hormone stimulation. Lipid peroxidation might also be important in the breakdown of excess smooth-surfaced endoplasmic reticulum in steroid-secreting cells. This would release cholesterol components of the membranes. Lipid peroxidation becomes especially interesting in light of the known competition between lipid peroxidation systems and microsomal hydroxylation systems for available NADPH (44, 46, 63). Such an interaction between lipid peroxidation, mediated by peroxisomal catalase, and microsomal hydroxylation could be involved in the control of steroid synthesis.

In addition to participation in steroid biosynthesis, peroxisomes may be involved in transport of steroids from one site to another along their complex synthetic path through the cell. Peroxisomes are widely distributed throughout the cell. They are seen adjacent to lipid droplets, among and continuous with the elements of endoplasmic reticulum as well as near mitochondria. The possible significance of this is not apparent in most steroid-secreting cells, where the other organelles are also more randomly organized. However, in mouse interstitial cells there is a rather clear-cut regional organization; mitochondria are located in areas where endoplasmic reticulum is sparse (18). In these cells peroxisomes are the organelles which cross regional lines and are found in both places (see Figs. 2, 6, [18]), thus providing a possible link between organelles involved in steroidogenesis.

All of these functional possibilities, NAD and NADPH generation, lipid peroxidation, and steroid transport, are intimately related to the functions of the smooth endoplasmic reticulum. The large number of peroxisomes seen in steroid-secreting cells and the continuity of these with the endoplasmic reticulum makes further study of peroxisomes in steroid-secreting cells a tantalizing prospect. However, intensive study both biochemical and experimental will be necessary to define their specific role.

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