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Association of isolated bovine kidney cortex peroxisomes with endoplasmic reticulum

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Close lateral membrane associations of peroxisomes with endoplasmic reticulum are a common feature in bovine kidney cortex epithelial cells. Isolated highly purified peroxisome preparations from this tissue showed a remarkable and persistent copurification of peroxisomal marker enzymes with small amounts (5%) of the microsomal reference enzymes esterase and glucose-6-phosphatase. Contamination with mitochondrial and lysosomal markers was negligible. Ultrastructural examination of such preparations revealed a peculiar association of vesicles or short tubular segments with the peroxisomal membrane. Short electron dense crossbridges seemed to maintain their structural association. The cytochemical localization of glucose-6-phosphatase in peroxisome-associated membrane structures confirmed their derivation from endoplasmic reticulum. The metabolic significance of such structural peroxisome-endoplasmic reticulum associations is discussed.

Introduction

The close association of peroxisomes with the membranes of the endoplasmic reticulum has been well known from ultrastructural studies in a variety of cell types (for review, see Refs. 1 and 2). In freeze-etch preparations of rat liver and kidney epithelial cells the cisternae of endoplasmic reticulum have been shown to cover large portions of the peroxisomal membrane [3,4]. In liver parenchymal cells the close contact between the two membranes has been reported to be maintained by electron dense intermembraneous crossbridges [5].

In early studies this close association seems to have been misinterpreted as luminal continuity (for review, see Ref. 6). The existence of a continuity gave rise to speculations concerning the biogenesis of peroxisomes from the endoplasmic reticulum, but recent electron microscopic studies have ruled out a luminal continuity [7]. The peculiar association thus might be rather the expression of a functional cooperation between these two compartments [8].

Recently a method for the isolation of highly purified peroxisomal preparations from mammalian liver [9] and kidney [10] was developed in our laboratory. In the course of these studies, it was noted that in the kidney of some species such as bovine and sheep a small amount of the microsomal marker enzymes esterase and glucose-6-phosphatase copurified consistently with the peroxisomal enzymes. Ultrastructural examination of such fractions revealed a striking association of smooth membrane elements with peroxisomes. The data for bovine kidney cortex peroxisomes which show this associations most conspicuously are presented in this paper.

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Material and Methods

Electron microscopy. Bovine kidneys obtained from the local slaughterhouse were excised shortly after exsanguination of animals and fixed by a 3 min perfusion through the renal artery. First physiological saline was introduced followed by 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6) containing 4% polyvinylpyrrolidone (PVP, Merck, Darmstadt). Adequately fixed kidney cortex tissue was dissected and immersed for 1 h in freshly prepared fixative. Sections of 50–100 μm obtained with an Oxford Vibratome were postfixed with the reduced osmium procedure of Karnovsky [11].

Peroxisome fractions obtained after gradient centrifugation were fixed by mixing with an equal volume of 1 or 5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6) and either filtrated onto Millipore filters according to the method of Baudhuin et al. [12] as modified by Völkl and Fahimi [9] or centrifuged at 25 000 × g for 10 min to form a pellet. Pellets were enclosed in 3% (w/v) agar. Tissue, pellicles and agar pellets were embedded in Epon 812.

Cytochemistry. Agar pellets of isolated peroxisome fractions fixed with low concentrations of glutaraldehyde (0.5% v/v) were incubated for 1 h (24° C) in a medium for the localization of glucose-6-phosphatase. The medium contained cerium chloride and glucose-6-phosphate as substrate [13]. In control experiments the substrate was omitted. Incubation was followed by postfixation in 1% aqueous osmium tetroxide.

Biochemistry. Peroxisomes from bovine kidney cortex were isolated as described recently [10]. Briefly, a fraction D [9,10] enriched in peroxisomes which was obtained by differential centrifugation of the homogenate was toploaded on a linear gradient of metrizamide (1.12–1.26 g/cm^3) and cell organelles were separated using density-dependent banding in a vertical rotor. Catalase (EC 1.11.1.6) and D-amino-acid oxidase (EC 1.4.3.3) were determined as described by Baudhuin et al. [14], urate oxidase (EC 1.7.3.3) as described by Priest and Pitts [15], esterase (EC 3.1.1.1) according to Beaufay et al. [16], glucose-6-phosphatase (EC 3.1.3.9) according to Baginski et al. [17], cytochrome c oxidase (EC 1.9.3.1) according to Cooperstein and Lazarow [18] and glucuronidase (EC 3.2.1.31) as described previously [9]. Protein was determined according to Lowry et al. [19]. The distribution of reference enzymes along the gradients are presented as histograms according to Beaufay et al. [20].

Results

Ultrastructural observations in situ

Peroxisomes are large and abundant in the epithelial cells of the proximal tubules of beef kidney cortex (Fig. 1). Most of them show an angular outline with flat straight portions of their membranes being underlaid with plate-like inclusions (marginal plates) (Figs. 1, 2). In addition, the majority of peroxisomes contain a polytubular core in their matrix (Figs. 1, 2). A frequent and consistent observation is the close association of segments of the endoplasmic reticulum with the limiting membranes of peroxisomes (Figs. 1, 2). Frequently such associations are found with the straight portion of the peroxisomal membrane (Figs. 1, 2). The side of endoplasmic reticulum membranes facing the peroxisomes are in all instances smooth with no ribosomes attached (Fig. 2). The membranes of endoplasmic reticulum and peroxisomes show a conspicuously parallel arrangement with a distance of approx. 10–15 nm (Fig. 2).

Ultrastructural observations of isolated peroxisomes

The isolated fractions consist of 97% peroxisomes with only few mitochondria and rare lysosomes (see also Ref. 10). A conspicuous finding is the association of membraneous elements consisting of smooth vesicles and tubules with the membranes of many peroxisomes (Fig. 3). The tubular segments have an average diameter of 30 nm and often form an anastomosing network with wide fenestrations around the peroxisomes (Fig. 4). The distance between the peroxisomal membrane and the tubular elements is approx. 10–15 nm. The intermembrane space is spanned by irregularly arranged thread-like electron densities (Fig. 5). No luminal continuities are observed.

Biochemical properties of isolated peroxisomes

The properties of purified peroxisome prepara-

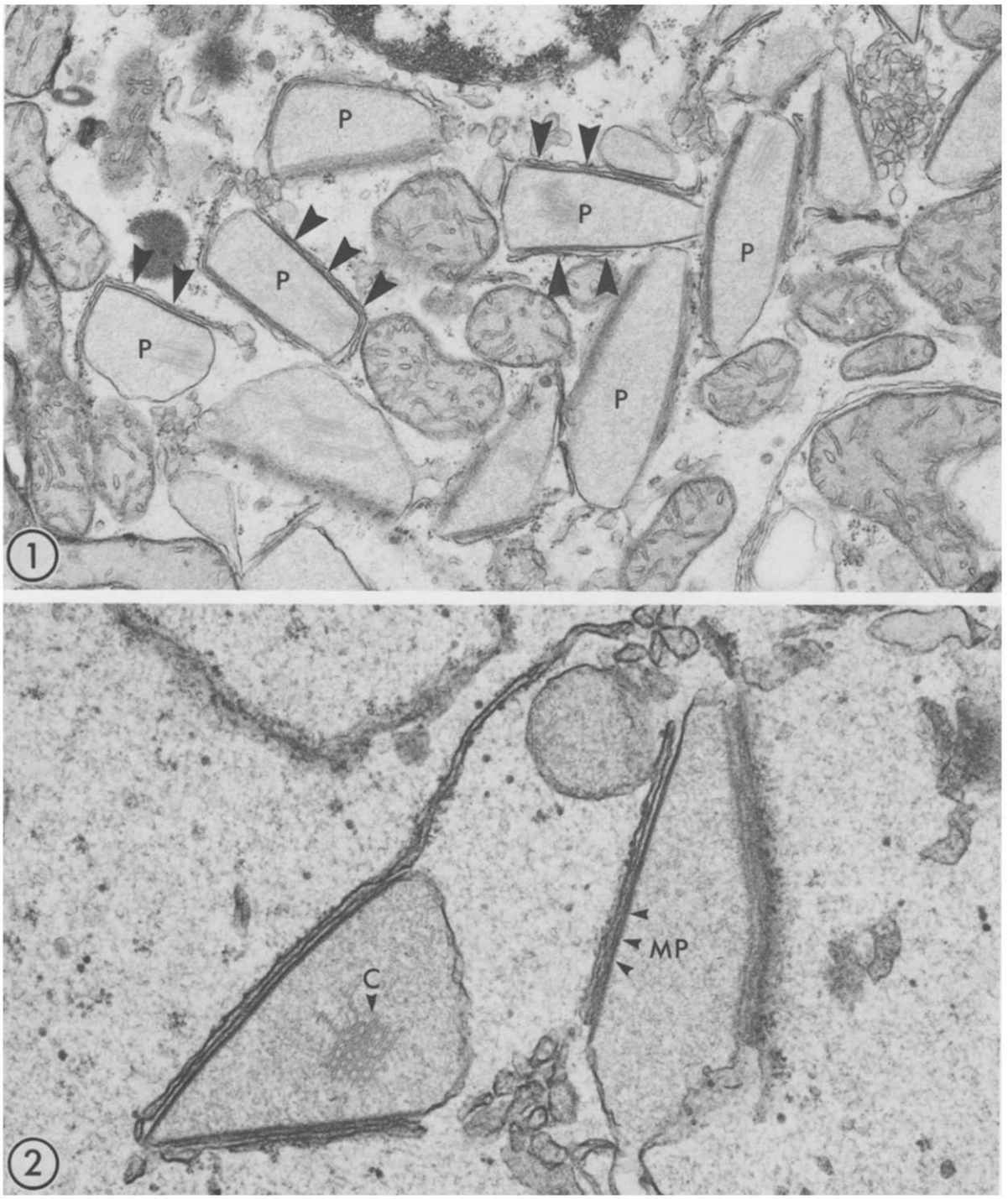


Fig. 1. Peroxisomes (P) are abundant in proximal tubule cells. They show often an angular outline. The flat straight portion is underlaid with marginal plates. Many peroxisomes contain a polytubular core. Segments of ER occur in close association with the membranes of peroxisomes (arrowheads). Magnification: ×32000.

Fig. 2. Peroxisome-endoplasmic reticulum associations at a higher magnification. Frequently such associations occur with the straight portions of the peroxisomal membrane. Membranes of peroxisomes and endoplasmic reticulum show a conspicuously parallel arrangement with a distance of only 10–15 nm. Marginal plate (MP), polytubular core (C). Magnification: ×59500.

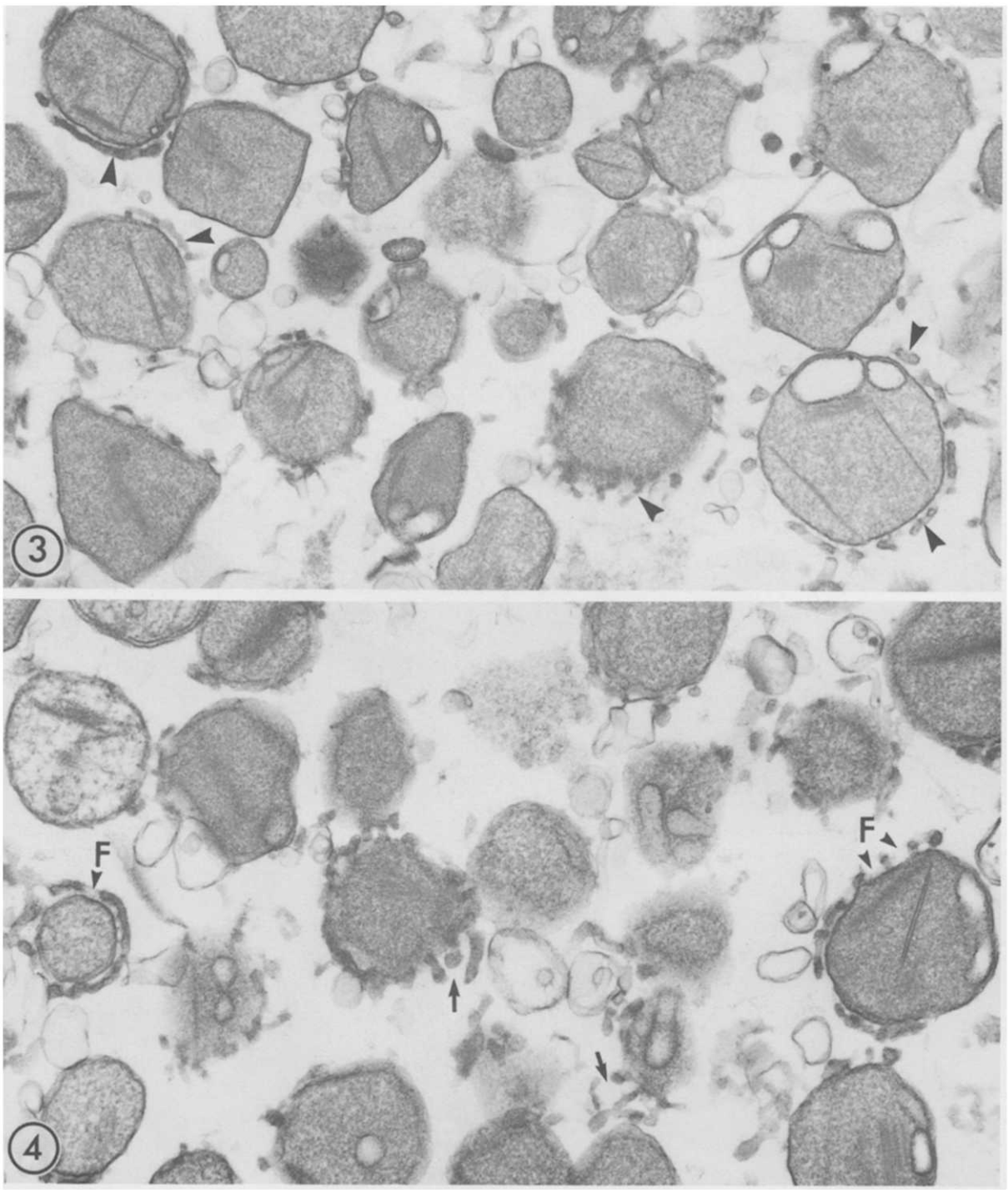


Fig. 3. View of an isolated peroxisome preparation. Some of the peroxisomes retain their angular shape. A conspicuous finding is the association of tubules and vesicles with the membranes of many peroxisomes (arrowheads). Magnification: ×38000.

Fig. 4. Tubular membrane segments with an average diameter of 30 nm often form a network with wide fenestrations (F) around peroxisomes. Particularily in grazing sections these tubules can be seen projecting from the peroxisomal surface (arrows). Magnification: ×48500.

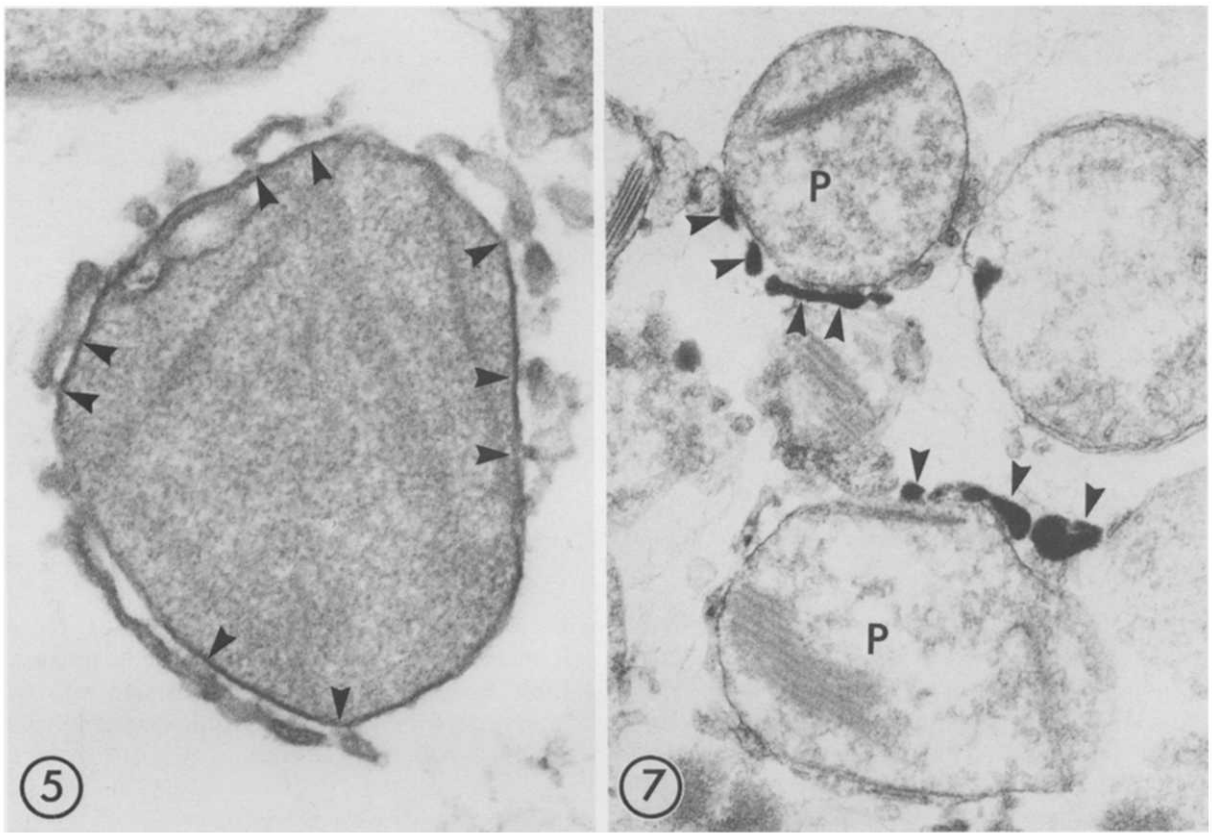
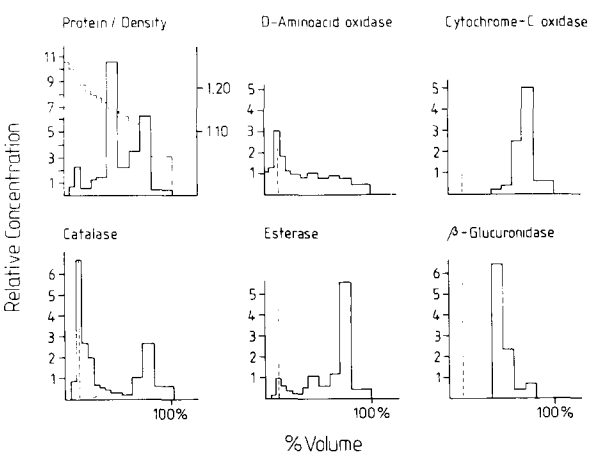


Fig. 5. The association of tubular membrane segments with the peroxisome surface at higher magnification. The distance between the two membranes is approx. 10–15 nm. The intermembrane space is spanned by irregularly arranged electron dense crossbridges (arrowheads). Magnification: ×97000.

Fig. 7. After cytochemical incubation for glucose-6-phosphatase, a microsomal marker enzyme, reaction product is demonstrated in the lumen of tubules and vesicles associated with peroxisomes (P) (arrowheads). The intensity of staining varies in different tubules and vesicles. Magnification: ×52000.



tions are compiled in Table I. On the average catalase activity is enriched 31 fold over that of the homogenate. Fig. 6 shows the distribution of organelle reference enzymes along the metrizamide gradient in a typical experiment. The per-

Fig. 6. Typical distribution profile of protein and marker enzyme activity in a metrizamide gradient after isopycnic density centrifugation for the isolation of peroxisomes. Peroxisomal reference enzymes are found in the bottom fractions and are well separated from mitochondrial and lysosomal markers. The bulk of the microsomal marker esterase is also well separated. However, a small amount of esterase activity forms a distinct peak corresponding to the peroxisomal markers.

TABLE I

COMPOSITION AND ENZYME ACTIVITIES OF PURIFIED PEROXISOME FRACTIONS FROM BOVINE KIDNEY CORTEX

Values are means of n experiments ± standard deviation.

	Homogenate (mU/mg protein)	Peroxisome fraction		
		(mU/mg protein)	Yield (%) [b]	RSA [c]
Catalase ($n = 11$)	225 ± 50	6970 ± 1200	7.7 ± 2.2	30.8
D-Amino-acid oxidase ($n = 7$)	3.6 ± 0.7	104 ± 5	6.6 ± 2.6	26.4
Urate oxidase ($n = 4$)	0.52 ± 0.3	40.5 ± 8.7	16.9 ± 5.6	67.6
Esterase ($n = 11$)	520 ± 230	1350 ± 790	0.53 ± 0.18	2.1
Glucose-6-phosphatase ($n = 4$)	76.2 ± 23.1 [a]	122.4 ± 36.4 [a]	0.35 ± 0.15	1.4
Cytochrome c oxidase ($n = 11$)	152 ± 55	64 ± 23	0.09 ± 0.045	0.36
β-Glucuronidase ($n = 11$)	26.3 ± 12	11 ± 10	0.11 ± 0.09	0.44
Protein (%) ($n = 11$)	100		0.25 ± 0.07	

[a] U/mg.
[b] Yield in % of the homogenate.
[c] RSA, relative specific activity (percentage recovered enzyme activity/percentage recovered protein, homogenate = 100%).

oxisomal marker enzymes catalase and D-amino-acid oxidase are recovered in the bottom fractions at an average density of 1.225 g/cm^3. Cytochrome c oxidase and β-glucuronidase the marker enzymes of mitochondria and lysosomes, respectively, band at lower densities (1.15 g/cm^3) and are well separated from the peroxisomal marker enzymes. Similarily, the bulk of esterase activity a marker enzyme for the endoplasmic reticulum compartment bands together with mitochondria and lysosomes (Fig. 6). In addition, however, a small amount of the esterase activity is recovered together with the peroxisomal reference enzymes (Fig. 6). This activity forms a distinct small peak separate from the bulk of esterase activity, thus ruling out a tailing down of the main microsomal fraction into the bottom peroxisomal fractions. Biochemical measurements revealed that on the average 5.6% of the esterase and 4.1% of the glucose-6-phosphatase activity toploaded on a gradient are recovered in the peroxisome fraction. Table II shows this data in comparison with recovery rates of peroxisomal, mitochondrial and lysosomal reference enzymes.

Cytochemistry of isolated peroxisomes

To learn more about the origin of peroxisome associated membrane vesicles and tubules we used a cytochemical, cerium based, procedure to demonstrate glucose-6-phosphatase a marker enzyme of the endoplasmic reticulum. After incubation, reaction product can be clearly demonstrated in the lumen of vesicles and tubules closely adhering to isolated peroxisomes (Fig. 7). No reaction product occurs within the peroxisomes. Interestingly, different vesicles and tubules associated with the same peroxisome sometimes show a complex staining behaviour. Neighbouring elements are

TABLE II

RECOVERY OF ENZYME ACTIVITIES IN PEROXISOME PREPARATIONS AFTER DENSITY DEPENDENT SUBFRACTIONATION OF PEROXISOME-ENRICHED D FRACTIONS IN METRIZAMIDE GRADIENTS

Values are given as % of D fraction activities. The number of experiments is as depicted in Table I.

	Catalase	D-Amino-acid oxidase	Urate oxidase	Esterase	Glucose-6-phosphatase	Cytochrome c oxidase	β-Glucuronidase
Peroxisome fraction	35.1 ± 6.9	27.8 ± 3.4	39.5 ± 12.7	5.6 ± 2	4.1 ± 1.6	0.23 ± 0.19	0.54 ± 0.73

either completely stained, show only some grains of reaction product or are without any detectable reaction product (Fig. 7).

Discussion

The data presented here clearly demonstrate the association of segments of endoplasmic reticulum with the membranes of peroxisomes in bovine kidney cortex. Such associations are seen not only in the in situ fixed kidneys but also in isolated peroxisome preparations obtained by differential and gradient centrifugation. The endoplasmic reticulum origin of membrane elements adhering to the isolated peroxisomes could be demonstrated by the cytochemical localization of glucose-6-phosphatase, an endoplasmic reticulum marker enzyme which is located in the membrane and releases its reaction product on the cisternal side of the membrane [8].

The ultrastructural and cytochemical observations are in full agreement with the biochemical findings that a small but distinct portion (5%) of the microsomal enzymes esterase and glucose-6-phosphatase copurify consistently with the peroxisomal fractions. A similar copurification has been observed in sheep but not in cat and rat renal cortex peroxisomes using the same isolation procedure. Such species differences suggest that peroxisome-endoplasmic reticulum complexes have a well defined physiological function which at the present time remains unknown.

The peroxisome-endoplasmic reticulum associations are obviously quite resistent to various manipulations in the course of the isolation procedure. The close proximity of peroxisomes and endoplasmic reticulum membranes seems to be maintained by electron dense intermembrane crossbridges of unknown nature. In 1974 Kartenbeck and Franke [5] called attention to similar crossbridges between peroxisomes and endoplasmic reticulum cisternae in hepatocytes and Morris hepatoma cells in situ. Such membrane to membrane crossbridges have been suggested to be a general means of membrane interaction and orientation [21].

No luminal continuities between endoplasmic reticulum segments and peroxisomes could be observed. This is in accordance with our finding that no diffusion of cytochemical glucose-6-phosphatase reaction product into peroxisomes could be observed confirming the in situ observations of Shio and Lazarow [8].

In rat liver tissue, mitochondria have also been reported to form intimate associations with the cisternae of endoplasmic reticulum (for review, see Refs. 22 and 23). Recently such complexes of Mito-rough endoplasmic reticulum have been isolated from rat liver and characterized biochemically [22,24,25]. It has been suggested that these complexes might serve as functional units for the assembly of microsomal cytochrome *P*-450b [26,27] or as preferential sites for the synthesis, cotranslational insertion and the transport of nuclear coded subunits of mitochondrial proteins [28,29]. In contrast to findings in mitochondria, however, the synthesis of peroxisomal proteins so far investigated occurs exclusively on free polysomes and proteins are inserted posttranslationally into peroxisomes (for review, see Ref. 6). An involvement of peroxisome-endoplasmic reticulum complexes in the synthesis and transport of nuclear coded peroxisomal enzymes, as proposed for mitochondria, thus appears unlikely.

A close spatial relationship between peroxisomes and endoplasmic reticulum, however, is a frequent observation in lipid synthesizing and secreting epithelial cells in certain sebaceous type glands [30,31]. Consequently this relationship might be an expression of the cooperation between the two compartments in the synthesis of complex lipids. Recent progress in purification procedures has revealed that peroxisomes contain a variety of enzymes engaged in lipid biosynthesis e.g. chain length specific fatty acyl-CoA synthetases [32–34], acyl-CoA : NADPH oxidoreductase involved in the generation of long chain fatty alcohols [35–37], as well as major enzymes of the dihydroxyacetone-phosphate pathway leading to ether-linked glycerolipids [38–40]. Peroxisomes, however, are not equipped with all the essential enzymes of the glycerolipid biosynthesis. The enzymes catalyzing the terminal reactions in the synthesis of glycerolipid and ether-linked glycerolipid occur in the microsomes (see, for example, Refs. 41–43). Thus, a shuttle of lipid intermediates between endoplasmic reticulum and peroxisomes might be required in the biosynthesis of complex lipids.

Other types of functional cooperation between the two compartments should also be considered. Recently, the localization of a dicarboxylate-CoA synthetase has been described in hepatic microsomes [44]. Dicarboxylic acid CoA's, however, are known to be oxidized in peroxisomes [45–47].

Investigations are now in progress to further characterize and clarify the functional significance of peroxisome-endoplasmic reticulum complexes.

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