

# Subcellular Localization of Sterol Carrier Protein-2 in Rat Hepatocytes: Its Primary Localization to Peroxisomes

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**Abstract.** Sterol carrier protein-2 (SCP-2) is a non-enzymatic protein of 13.5 kD which has been shown in *in vitro* experiments to be required for several stages in cholesterol utilization and biosynthesis. The subcellular localization of SCP-2 has not been definitively established. Using affinity-purified rabbit polyclonal antibodies against electrophoretically pure SCP-2 from rat liver, we demonstrate by immunoelectron microscopic labeling of ultrathin frozen sections of rat liver that the largest concentration of SCP-2 is inside peroxisomes. In addition the immunolabeling indicates that there are significant concentrations of SCP-2 inside mitochondria, and associated with the endoplas-

mic reticulum and the cytosol, but not inside the Golgi apparatus, lysosomes, or the nucleus. These results were confirmed by immunoblotting experiments with proteins from purified subcellular fractions of the rat liver cells carried out with the anti-SCP-2 antibodies. The large concentration of SCP-2 inside peroxisomes strongly supports the proposal that peroxisomes are critical sites of cholesterol utilization and biosynthesis. The presence of SCP-2 inside peroxisomes and mitochondria raises questions about the mechanisms involved in the differential targeting of SCP-2 to these organelles.

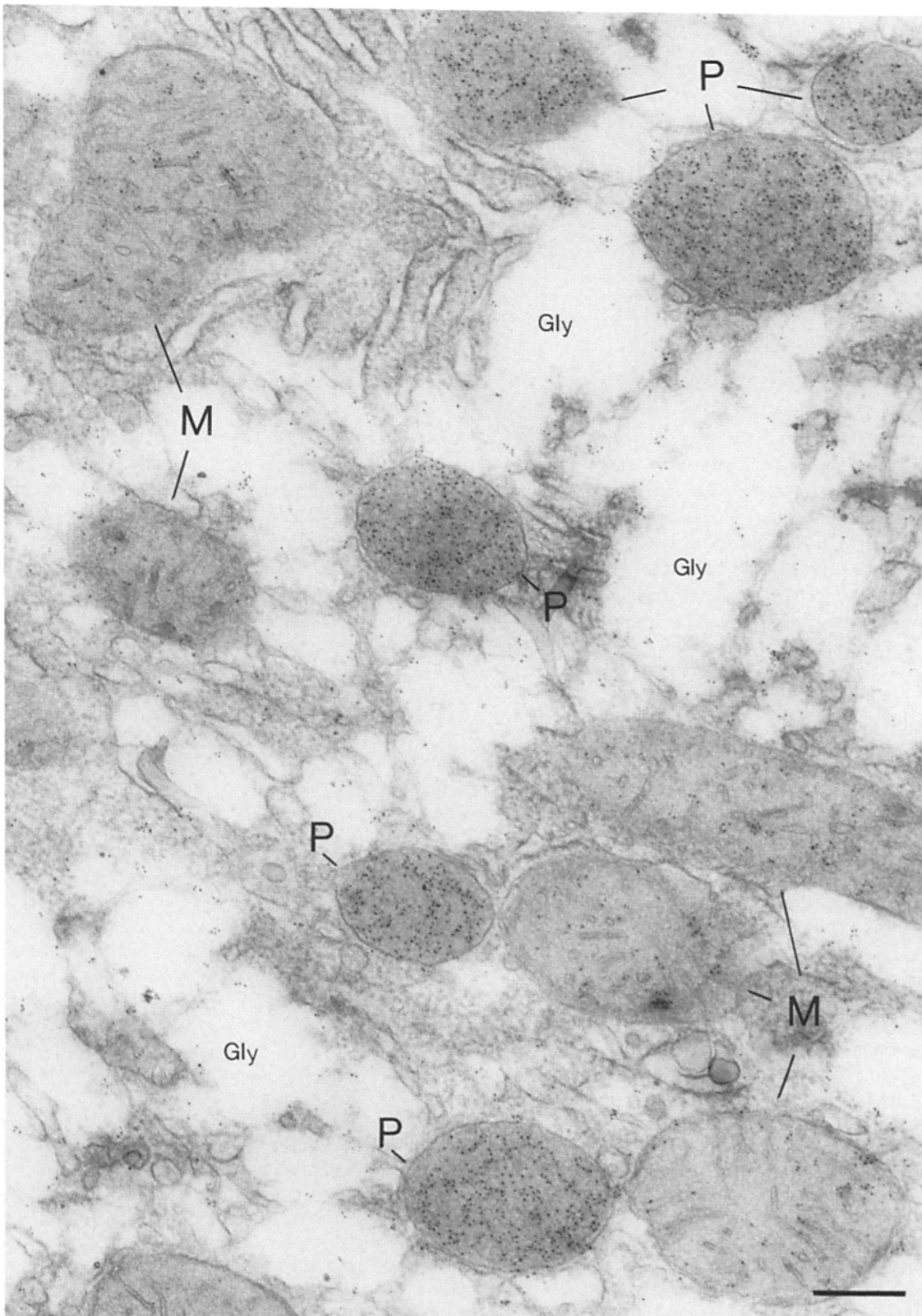
**P**EROXISOMES in higher eukaryotic cells have been implicated as critical sites of cholesterol metabolism. Peroxisomal fractions obtained from rat liver can oxidize  $3\alpha,7\alpha,12\alpha$ -trihydroxy- $5\beta$ -cholestanoic acid, an intermediate product of cholesterol metabolism, to cholic acid (11) and can convert cholesterol to propionic acid (8). Highly purified (95–98%) rat liver peroxisomes are also able to oxidize 26-hydroxycholesterol, the product of the enzymatic C-26 hydroxylation of cholesterol, to a C-24 bile acid (17). The physiological significance of peroxisomal bile acid synthesis is illustrated by the observation that in peroxisomal deficiency diseases (Zellweger's syndrome) there is an accumulation of bile acid intermediates due to defective peroxisomal cleavage of the C-27 steroid side chain (12). Peroxisomes may also play an important role in cholesterol biogenesis. It has been demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme of the sterol biosynthetic pathway, is present not only in the membranes of the endoplasmic reticulum but also in peroxisomes. Immunoelectron microscopic labeling of ultrathin frozen sections of normal liver, using two monoclonal antibodies to purified rat liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase, illustrated that the enzyme is in fact present at a considerably larger concentration

in the matrix of peroxisomes than in the endoplasmic reticulum (14). In a subsequent study, it was determined that between 20 and 30% of the total 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (based on biochemical data) was located in the peroxisomes of cholestyramine-treated animals (15). In addition, it has recently been shown that rat liver peroxisomes in the presence of cytosolic proteins *in vitro* are able to convert  $^{14}\text{C}$ -mevalonic acid to  $^{14}\text{C}$ -cholesterol (31).

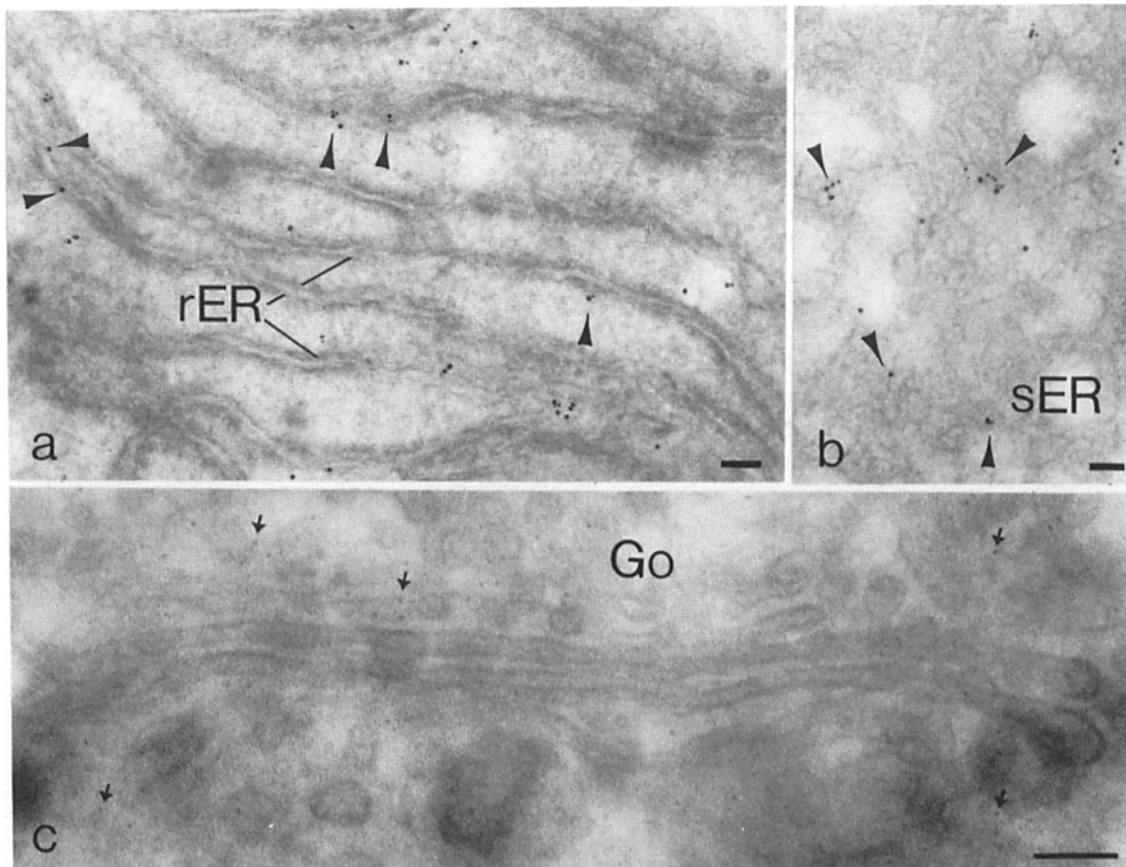
It is of interest, therefore, to inquire whether other proteins thought to be involved in cholesterol utilization or biogenesis are also present in peroxisomes. In this study, we have investigated the subcellular localization of sterol carrier protein-2 (SCP-2).<sup>1</sup> This nonenzymatic protein has a variety of carrier functions *in vitro* all associated with cholesterol biochemistry. SCP-2 has been shown to be required in the enzymatic conversion of lanosterol to cholesterol during cholesterol biogenesis by rat liver microsomal enzymes (26), in the use of cholesterol via esterification by acyl-coenzyme A cholesterol acyltransferase (6), and in the synthesis of microsomal and mitochondrial bile acid (2). SCP-2 is also involved in the intracellular transfer of cholesterol which is required for pregnenolone production in adrenal (2, 34, 35) and ovarian tissues (3). As purified from a rat liver cytosol preparation (26), SCP-2 is a soluble protein of 13.5 kD and an isoelectric

A preliminary account of this work has appeared in abstract form (1987. *J. Cell Biol.* 105[5, Pt. 2]:157a. [Abstr.]).

1. *Abbreviation used in this paper:* SCP-2, sterol carrier protein-2.



**Figure 1.** An electron micrograph of an ultrathin frozen section of normal rat liver indirectly immunolabeled with rabbit polyclonal antibodies to SCP-2 followed by colloidal gold adducts of guinea pig antibodies to rabbit IgG. The peroxisomes (*P*) are intensely and uniformly immunolabeled. The mitochondria (*M*) exhibit a lower level of labeling, as does the cytosol (as seen at the boundaries of the glycogen fields [*Gly*]). Bar, 0.5  $\mu\text{m}$ .



**Figure 2.** Electron micrographs of fields similar to that of Fig. 1 exhibiting immunolabeling (*arrowheads*) for SCP-2 over different intracellular compartments: (a) the rough endoplasmic reticulum (*rER*), (b) the smooth endoplasmic reticulum (*sER*), and (c) the Golgi apparatus (*Go*). Note the absence of immunogold labeling over the Golgi apparatus; the short arrows indicate the endogenous ferritin particles. Bars, 0.1  $\mu\text{m}$ .

point of 8.6. It has been sequenced (24, 28, 39) and shown to be identical to proteins isolated by several groups (25, 29, 33). The subcellular distribution of SCP-2 in rat liver was previously studied using polyclonal antibodies to SCP-2 (37); immunogold localization was reported on peroxisomes and in the cytosol, but the immunoblotting of the proteins of a peroxisomal fraction showed that the same antibodies labeled only a 58-kD rather than a 13.5-kD band. In the absence of further characterization of the 58-kD protein, whether the antibodies were truly monospecific for SCP-2, or whether they also contained antibodies directed to a peroxisomal protein of 58 kD that was unrelated to SCP-2, was therefore not resolved.

In this study, we have investigated the localization of SCP-2 inside rat hepatocytes by two methods: (a) immunoelectron microscopic labeling of ultrathin frozen sections of whole cells, using monospecific affinity-purified polyclonal antibodies to SPC-2; and (b) immunoblotting of extracts of purified subcellular fractions of the fragmented cells with the same antibodies. Both methods gave consistent results. They showed that the largest concentration of SCP-2 is present in peroxisomes, but that significant concentrations are also present inside mitochondria and associated with the endoplasmic reticulum.

## Materials and Methods

### Animals

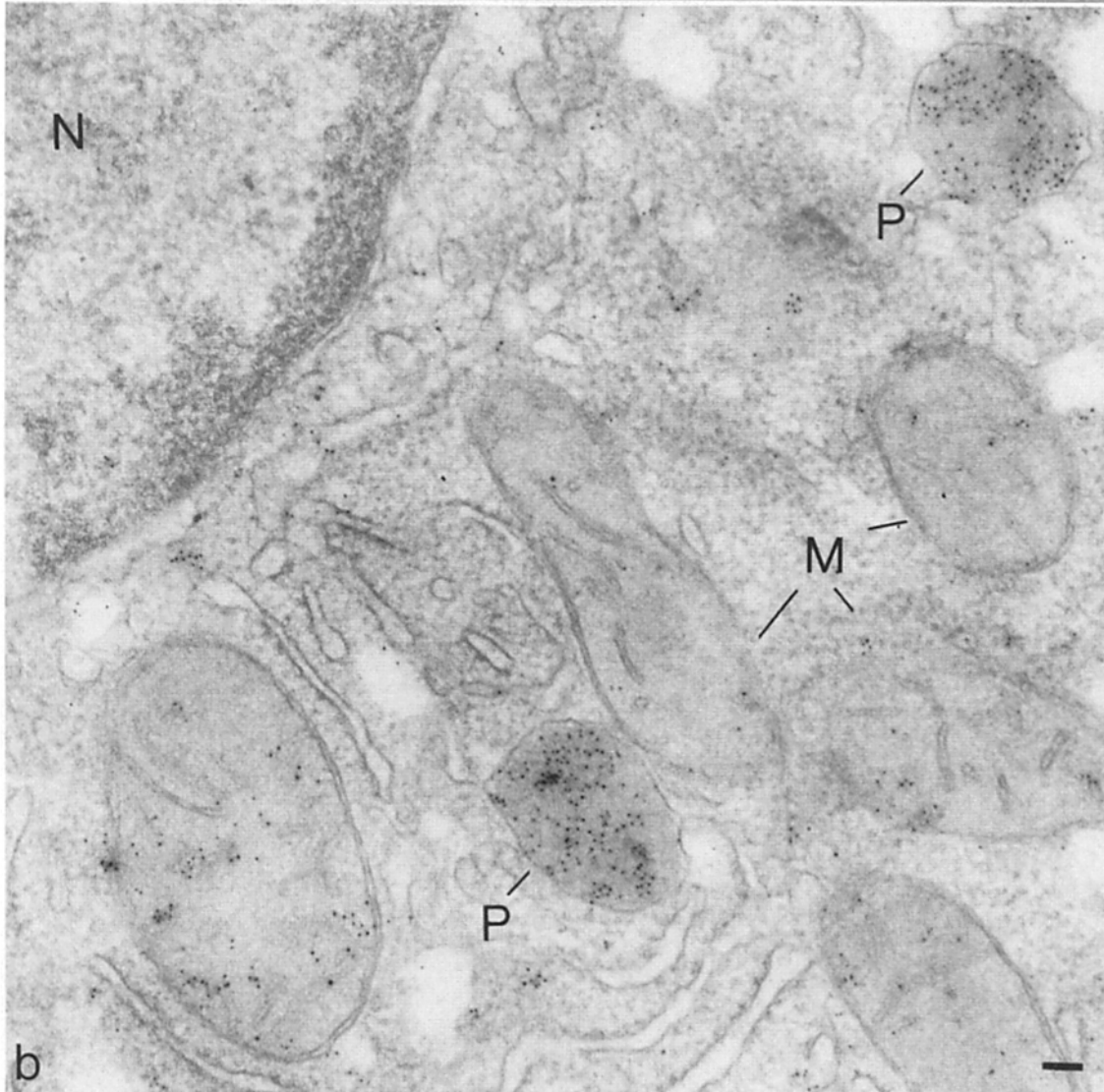
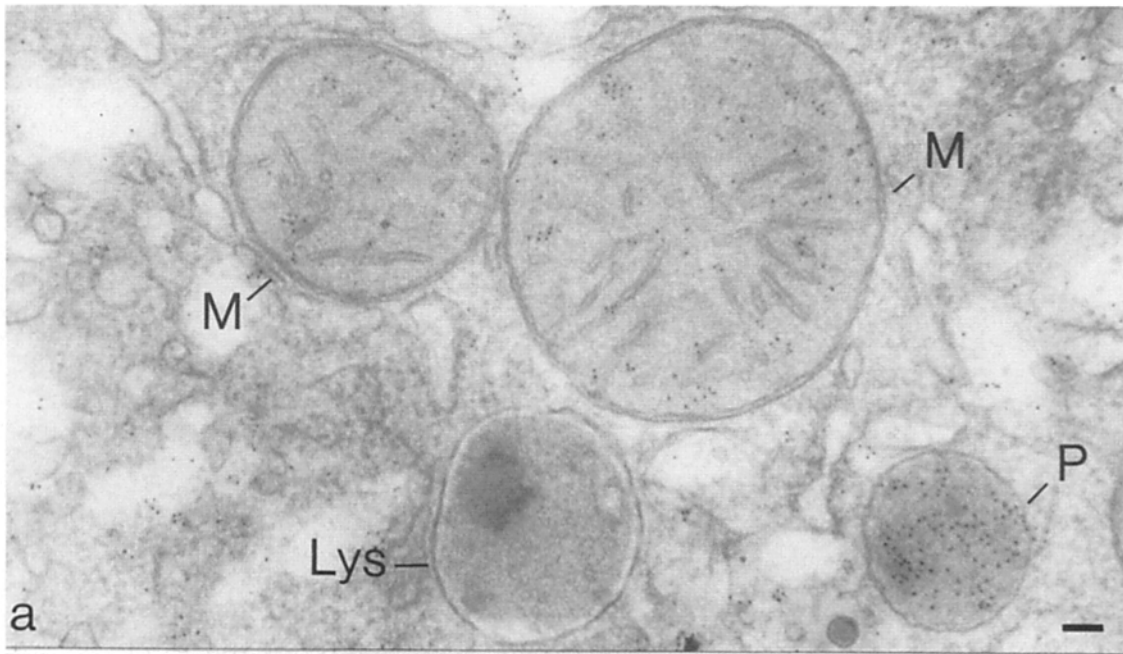
Male Sprague-Dawley rats were kept in a room from which external illumination was excluded. Light and dark periods (12 h each) were regulated by electric timers; the light was turned off at 6 PM and turned on at 6 AM. The animals had access to food and water at all times. All rats (average weight 150–180 g) were fasted for 12 h and killed by decapitation.

### Purification of SCP-2 and Preparation of Antibodies

SCP-2 was purified from rat liver by the method of Scallen et al. (30) and affinity-purified rabbit polyclonal antibodies against the cytosolic SCP-2 were prepared as described (28, 30). The monospecificity of these antibodies for SCP-2 is demonstrated in the papers cited and also by the immunoblotting results described in Figs. 5 and 6. The IgG fraction of rabbit antibodies to bovine liver catalase, which cross react with other mammalian catalases (16), was the gift of A. Schram (University of Amsterdam).

### Immunoelectron Microscopy

Livers from rats were perfused for 5 min through the ascending aorta with a fixative solution containing 3% paraformaldehyde plus 0.5% glutaraldehyde in cacodylate buffer, pH 7.4. Small blocks of fixed liver were then kept in the same fixation solution for 2 h. Cryoultramicrotomy was performed according to Tokuyasu (32). The tissue blocks were infused with 2.3 M sucrose for 30 min, frozen in liquid nitrogen, and sectioned at  $-90^{\circ}\text{C}$  with a cryoultramicrotome. Ultrathin frozen sections were transferred onto



parlodion-covered nickel grids and immunolabeled with affinity-purified rabbit polyclonal antibodies against the cytosolic SCP-2. The primary antibodies were used at a concentration of 5 µg/ml. After washing, the sections were treated with colloidal gold (6–8-nm-diam) adducts of guinea pig antibodies to rabbit IgG (13). Immunolabeled frozen sections were osmicated, poststained with 0.5% uranyl acetate in barbital/acetate buffer, pH 5.2, dehydrated in a series of ethanol solutions to pure ethanol, and infused in LR white acrylic resin (Polysciences, Inc., Warrington, PA). After infusion, polymerization was effected in a microwave oven for 3 min or overnight in a vacuum oven at 60°C (13). The grids were observed in a transmission microscope (model 300; Philips Electronic Instruments, Inc., Mahwah, NJ) at 80 kV without any poststaining.

### **Isolation of Peroxisomes, Microsomes, and Mitochondria**

Liver homogenates were fractionated as described by Leighton et al. (20) except that preinjection of rats with Triton WR-1339 was omitted and the peroxisome-enriched fraction (which also contained lysosomes, mitochondria, and microsomes) was washed only once. The peroxisome-enriched fraction was then further fractionated by equilibrium density centrifugation on a steep linear metrizamide gradient (9). The gradient was centrifuged (model OTD 75B; Sorvall Instruments Div., Newton, CT) using an ultravertical rotor (model TV 850) at 40,000 rpm for 50 min at 8°C. A total of 20–25 fractions were collected from the bottom of the centrifuge tube with a two-way needle. The mitochondrial and peroxisomal fractions of greatest purity obtained from the gradient were used for the biochemical and immunoblotting assays. Rat liver microsomes were prepared by differential centrifugation (4), resulting in a microsomal and soluble fraction. All cell fractions were assayed for protein content and distribution of marker enzyme activities.

### **Assay of Marker Enzymes**

The activities of catalase (a peroxisomal marker) and cytochrome oxidase (a mitochondrial marker) were measured according to Leighton et al. (20) and Lazarow and de Duve (19). Esterase (a microsomal marker) was measured according to Beaufay et al. (1). Protein was determined by the method of Lowry et al. (22) using BSA as a standard. Since metrizamide interferes with the determination of protein, aliquots of the gradient samples were first precipitated in 10% TCA.

### **SDS-PAGE and Immunoblotting**

The proteins of the peroxisomal, mitochondrial, microsomal, and soluble fractions were separately fractionated on 12.5% polyacrylamide slab gels containing 0.1% SDS, according to Laemmli (18). Electrophoresis was performed at 35 mA/gel. Protein samples were prepared in solubilizing buffer (0.125 M Tris/HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol, 5% glycerol, and 0.005% bromophenol blue) and incubated for 20 min in a 37°C water-bath before loading the gel. A mixture of molecular weight standards was routinely applied to the gel. The separated proteins were electrophoretically transferred to nitrocellulose paper in 20 mM Tris, 150 mM glycine, and 20% methanol. The nitrocellulose was incubated with gentle shaking for 60 min at 37°C with 5% BSA in Tris-buffered saline, pH 7.4, overnight at 4°C with polyclonal antibody against rat liver cytosolic SCP-2, and then for 60 min with horseradish peroxidase-conjugated goat anti-rabbit IgG.

### **Purification of Antibodies Using Antigens Immobilized on Nitrocellulose**

Purified peroxisomal fractions (115 µg peroxisomal protein/well) were solubilized in sample buffer containing 2.5% SDS and 2% β-mercaptoethanol. The protein components were separated on a 10% polyacrylamide gel. After electrophoresis, the proteins were transferred to nitrocellulose and stained with Amido black. The bands at 13.5 kD and 55 kD were 4.5 cm apart on

the nitrocellulose. They were cut out in narrow strips and placed in 6-cm petri dishes. The strips were blocked for 2 h in 3% BSA in Tris-buffered saline and incubated overnight with polyclonal antibodies against SCP-2 (5 µg/ml in 3% BSA/Tris-buffered saline). After extensive washing in Tris-buffered saline, the antibody was eluted from each strip (27) with 1 ml of 0.2 M HCl-glycine, pH 2.4, for 40 min and then immediately dialyzed against 10% PBS containing an equivalent concentration of Tris base. The eluted antibody was then lyophilized, resuspended in 100 µl water, and used for the immunoelectron microscopic labeling experiments shown in Fig. 3 and the immunoblotting experiments shown in Fig. 7.

### **Materials**

Horseradish peroxidase-conjugated goat anti-rabbit IgG antibodies were obtained from Bio-Rad Laboratories (Richmond, CA). Other chemicals were from Sigma Chemical Co. (St. Louis, MO).

## **Results**

### **Immunoelectron Microscopy**

Indirect immunolabeling using affinity-purified rabbit polyclonal antibodies against rat cytosolic SCP-2 was carried out on ultrathin frozen sections from normal rat liver. A representative electron micrograph is shown in Fig. 1. The interiors of the peroxisomes are uniformly and intensely labeled with gold particles. The mitochondria are also labeled inside, but at a substantially lower intensity than the peroxisomes. The immunolabels inside the mitochondria often appeared to be associated with the inner membrane (Fig. 1; see also Fig. 3). In addition, the endoplasmic reticulum region (Fig. 2, *a* and *b*) showed low intensity, but definitely positive, labeling. Closer examination of many immunolabeled endoplasmic reticulum fields suggested that the labels were often bound to the membrane, predominantly on the cytoplasmic surface. The cytosol was also labeled (visible in Fig. 1 at the periphery of the glycogen fields). On the other hand, no significant labeling for SCP-2 above background could be detected in the Golgi apparatus (Fig. 2 *c*), lysosomes (Fig. 3 *a*), or the nucleus (Fig. 3 *b*).

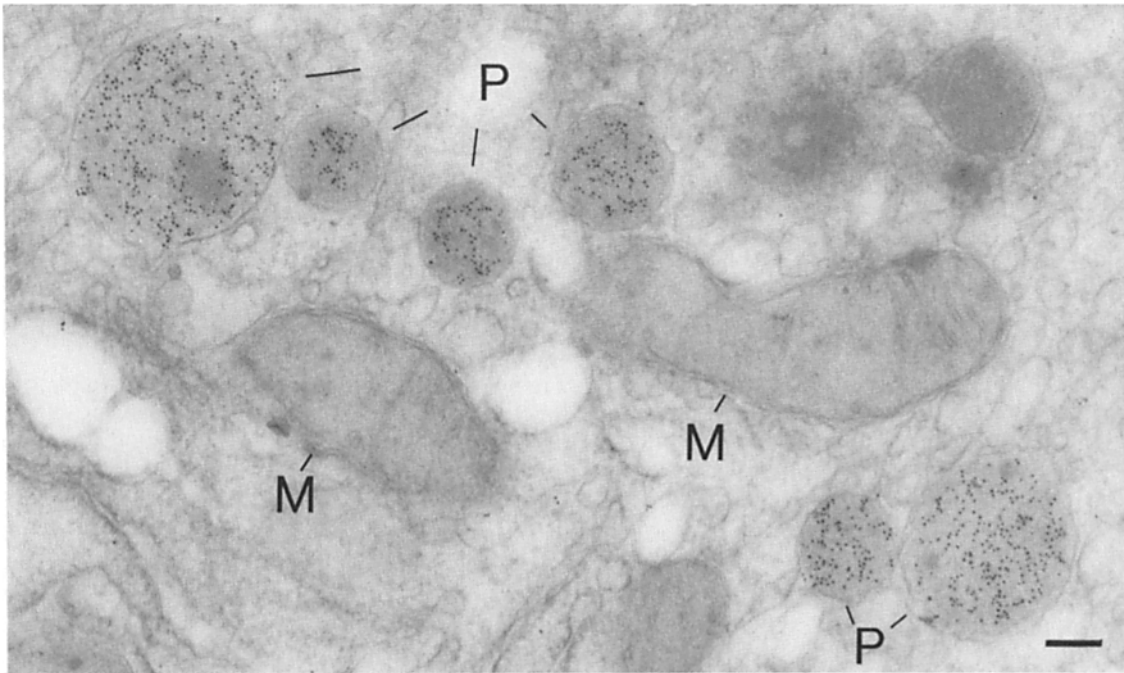
For comparison with these results, Fig. 4 shows similar sections labeled with polyclonal antibodies to the exclusively peroxisomal protein catalase. Intense immunolabeling of the peroxisomes is observed, but the mitochondria are not detectably labeled as expected. This is one indication that the low level of labeling for SCP-2 in the mitochondria (Fig. 1 and Fig. 3) and associated with the endoplasmic reticulum (Fig. 2, *a* and *b*) is specific.

### **Immunoblotting of Cell Fractions**

The purity of the peroxisomal, mitochondrial, microsomal, and soluble fractions was determined as described in the Materials and Methods. The peroxisomal fractions used were ~95% pure based on the measurements of marker enzymes (5). The only significant contaminant was the microsome fraction; the activity of the marker enzyme for mitochondria (cytochrome oxidase) was below the level of

**Figure 3.** Electron micrographs of fields similar to those of Figs. 1 and 2. (*a*) Immunolabeling with antibodies specifically absorbed to and eluted from the 13.5-kD band of peroxisomal proteins; and (*b*) immunolabeling with antibodies from the 55-kD band. Note that the labeling results are indistinguishable. Additional examples are shown of immunolabeling for SCP-2 of peroxisomes (*P*) and mitochondria (*M*), but note the absence of labeling from lysosomes (*Lys*) and from the nucleus (*N*). Bars, 0.1 µm.





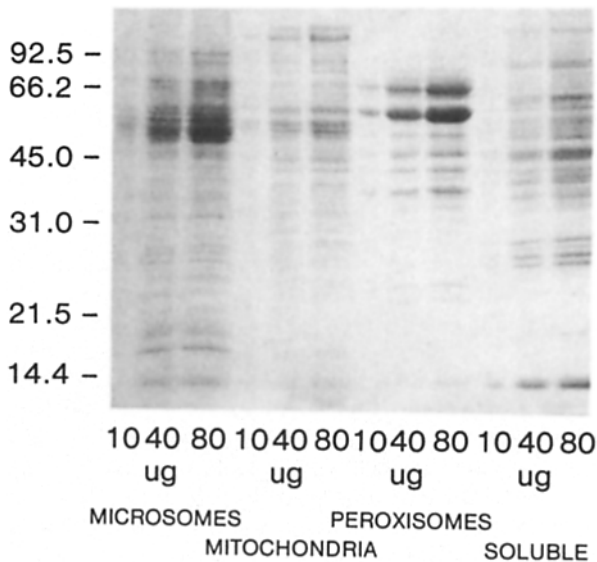
**Figure 4.** An electron micrograph of an ultrathin section of normal rat liver indirectly immunolabeled with rabbit polyclonal antibodies to catalase followed by colloidal gold adducts of guinea pig antibodies to rabbit IgG. The peroxisomes (*P*) are intensely and uniformly immunolabeled but the mitochondria (*M*) show no detectable labeling. Bar, 0.5  $\mu$ m.

detection (<1% mitochondrial contamination). The mitochondrial fraction contained 12% microsomal and 5% peroxisomal contaminants. The microsomal fraction contained ~3% mitochondrial and only 0.4% peroxisomal protein. The soluble fraction was contaminated with 3% microsomal and 4% peroxisomal proteins.

The Coomassie Blue staining patterns for the proteins of these fractions separated on SDS-PAGE are shown in Fig. 5. The corresponding immunoblots made with the polyclonal anti-SCP-2 antibodies of these samples transferred to nitrocellulose are reproduced in Fig. 6. They show that a band at the molecular mass of 13.5 kD corresponding to SCP-2 was the predominantly immunolabeled protein. This labeled band was clearly most prominent in the peroxisomal fraction. Another band at 55 kD was also labeled with the antibodies in several fractions; a band of similar molecular mass was immunoblotted in previous studies with polyclonal anti-SCP-2 antibodies (37). It was essential to determine whether the 55-kD band represented a form of SCP-2 or some unrelated contaminant. Accordingly, antibodies absorbed by the 55- and 13.5-kD bands were separately eluted (27). Each was used in immunoelectron microscopic labeling experiments and in immunoblotting a peroxisomal fraction as described in the Materials and Methods. The antibodies isolated from the 13.5- and 55-kD band yielded identical immunolabeling results (Fig. 3, *a* and *b*, respectively). Each isolated antibody blotted both the 13.5- and 55-kD bands in the peroxisomal fraction (Fig. 7). The two sets of results clearly demonstrate that the same antibodies recognize both the 13.5- and 55-kD proteins. This has been confirmed subsequently by the finding that a monoclonal antibody to the 13.5-kD protein immunoblotted both the 13.5- and 55-kD bands (Krisans, S., and T. J. Scallen, unpublished results).

## Discussion

We have demonstrated, using affinity-purified polyclonal antibodies raised against electrophoretically pure SCP-2 from rat liver, that by far the largest concentration of SCP-2 inside the hepatocytes is in peroxisomes. This conclusion was obtained from immunoelectron microscopic labeling experiments carried out with these antibodies (Figs. 1–3) and then confirmed by immunoblotting experiments of the proteins of purified peroxisomal and other subcellular fractions using the same anti-SCP-2 antibodies (Figs. 5 and 6). One complication observed in the immunoblotting experiments was the labeling not only of the expected protein band at 13.5 kD but also of a band at 55 kD. In previous experiments (37), a band corresponding to the 55-kD protein was the only immunoreactive component in peroxisomal extracts, but the identity of that component and its relationship to the 13.5-kD protein was not established. It was possible, for example, that different antibody species in the polyclonal antibodies raised to the 13.5-kD protein recognized unrelated 13.5- and 55-kD proteins. We showed, however, that antibodies specifically reacting with either of these two protein bands yielded indistinguishable immunoelectron microscopic labeling results (Fig. 3, *a* and *b*) and, furthermore, each antibody blotted both bands (Fig. 7). The simplest interpretation of these results is that the two bands represent two different states of aggregation (monomer and tetramer) of the same protein. It has not been ruled out, however, that the 13.5-kD protein is a subunit of a 55-kD protein encoded by another gene. In any event, our results resolve the previous ambiguity (37) and show that identical or closely related species of SCP-2 molecules are present in peroxisomes and the several other intracellular sites discussed below.



**Figure 5.** Electrophoresis on 0.1% SDS/12.5% polyacrylamide gels and Coomassie Blue staining of the proteins of the several intracellular fractions indicated; three different amounts of each protein fraction were applied to the gel as noted. The Coomassie labeling reveals the distinctive and complex protein patterns of each fraction.

The predominant localization (but not exclusive localization; see below) of SCP-2 to peroxisomes inside hepatocytes strongly supports a preeminent role for peroxisomes in cholesterol biochemistry. As detailed in the introduction, SCP-2 has been critically implicated by *in vitro* experiments in carrier functions in both cholesterol utilization and biosynthesis. It appears that SCP-2 is necessary to transfer certain intrinsically water-insoluble cholesterol precursors and metabolic products between one component and the next in a cascade of enzyme reactions. Many of these cholesterol-related carrier functions of SCP-2 must therefore be taking place inside peroxisomes.

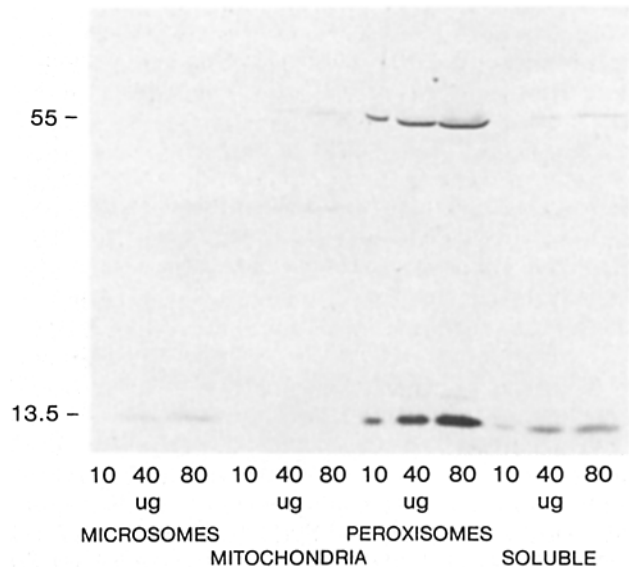
That peroxisomes are the major sites of localization of SCP-2 is also consistent with the recent finding (36) that human SCP-2 is present in substantially reduced amount in the livers of infants with cerebro-hepato-renal (Zellweger) syndrome, a condition in which hepatic peroxisomes are largely absent.

In addition to the predominant peroxisomal localization of SCP-2, lower concentrations of SCP-2, or an immunologically indistinguishable protein, were also found inside mitochondria (Figs. 1 and 3) and associated with the endoplasmic reticulum (Figs. 1 and 2) and the cytosol. However, there was no detectable labeling in the Golgi apparatus, lysosomes, or nuclei. The labeling for SCP-2 in the mitochondria was often observed to be concentrated on the inner mitochondrial membranes. As one control for this mitochondrial labeling for SCP-2, we observed that immunoelectron microscopic labeling for catalase is absent from mitochondria (Fig. 4), corresponding to the exclusively peroxisomal localization of that protein. In other words, the background of nonspecific labeling achieved by our immunoelectron microscopic methods, as demonstrated with labeling for catalase, is substantially lower than the observed labeling of mitochondria for SCP-2. The latter is therefore specific. The SCP-2 labeling of the endoplasmic reticulum (Figs. 1 and 2) appears to be

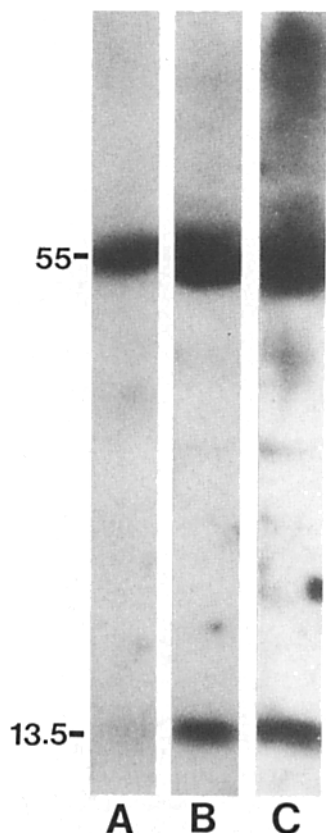
largely associated with the cytoplasmic face of the reticulum membranes and may, therefore, reflect a peripheral membrane association of the soluble cytosol form of SCP-2. This is supported by the observation that when the endoplasmic reticulum fraction is treated with  $\text{Na}_2\text{CO}_3$  (5), immunoblotting of the membrane-associated and solubilized proteins shows that SCP-2 is exclusively found in the soluble fraction (data not shown). A peripheral cytoplasmic association is consistent with the absence of SCP-2 labeling of the Golgi apparatus (Fig. 2 c). If the SCP-2 associated with the endoplasmic reticulum was present in the reticulum lumen, one might have expected it to have been transferred in part to the Golgi apparatus along the secretory pathway.

The association of low concentrations of SCP-2 with several intracellular sites other than peroxisomes is also observed in the immunoblotting experiments with the proteins of several purified subcellular fractions of the rat liver cells (Figs. 5 and 6), although the level of peroxisomal contamination (5%) of the mitochondrial fraction presents a problem. The electron microscopic immunolabeling results, however, are definitive regarding a mitochondrial SCP-2.

In addition to sequences of enzymatic reactions all occurring within a cellular compartment (e.g., peroxisomes) which may involve transfers of substrates and products via the endogenous SCP-2 at various stages, there may also be sequences of reactions in which the early steps occur in one intracellular compartment (e.g., the cytosol) and later steps in another compartment (e.g., the mitochondria). This could be one of the reasons to have SCP-2 present in different compartments. We suggest that since sterol-bound SCP-2 molecules are not likely to get across intact membranes as such, the transfer of the particular sterol from one compartment to another (say from the cytosol to mitochondria) may involve



**Figure 6.** Immunoblots with antibodies to SCP-2 of transfers from electrophoresis gels, such as those of Fig. 5, followed by treatment with horseradish peroxidase-conjugated secondary antibodies. Bands at 13.5 and 55 kD are the only ones that are immunolabeled in each of the fractions. The labeling of the peroxisomal fraction clearly greatly exceeds that of the other fractions. At larger concentrations of protein the bands at 13.5 and 55 kD in the mitochondrial fraction are more prominent (not shown).



**Figure 7.** Immunoblots of a peroxisomal extract with antibodies eluted (27) from (A) the 13.5-kD band and from (B) the 55-kD band of a peroxisomal gel fractionation transfer. In C, the unfractionated anti-SCP-2 antibodies were used as a control. Note that each of the specifically absorbed and eluted antibodies in A and B reacts with both bands. These eluted antibodies were the same as used in the immunolabeling experiments of Fig. 3.

(a) binding of the sterol to cytosolic SCP-2, (b) binding of the sterol-bound SCP-2 to the mitochondrial outer membrane, (c) the release of the sterol to the membrane and its diffusion across the membrane, and then (d) the uptake of the sterol by SCP-2 molecules inside the mitochondrion.

The existence of several intracellular locations of SCP-2 raises interesting questions about the different processes required to target SCP-2 to these different sites. In particular, for a protein like SCP-2 encoded by a nuclear gene and synthesized in the cytosol, two different types of signal sequences are required for targeting to peroxisomes and to mitochondria. For peroxisomal targeting, the signal appears to be located near the carboxy terminus of the polypeptide chain (7); a carboxy-terminal tripeptide sequence -ser-lys-leu is often, but not exclusively, associated with peroxisomal entry (Gould, S. J., G.-A. Keller, and S. Subramani, personal communication). Mitochondrial targeting, on the other hand, is associated with one of several kinds of signal sequences located at the amino terminus of the polypeptide chain. This signal sequence is often cleaved off the chain after entry into the mitochondrion to form the mature protein. The cDNA for mouse liver SCP-2 has been produced and its nucleotide sequence determined (23); this work will be reported in detail elsewhere. It is interesting, however, to briefly note two features of the amino acid sequence predicted from the cDNA: (a) the carboxy-terminal sequence is -ala-lys-leu, whereas the carboxy terminus of the isolated mature rat liver SCP-2 has been reported variously as -ala-lys, missing a terminal leu (28), or as ala-lys-leu (24); and (b) a 20-amino acid amino-terminal sequence that is missing

from the mature protein. An -ala-lys-leu carboxy terminus might serve as a peroxisomal targeting sequence (7), while the apparent susceptibility of the terminal leu residue to proteolytic cleavage could result in eliminating that function. The 20-amino acid amino-terminal sequence does not resemble any of the known cleavable signal sequences for the endoplasmic reticulum because it lacks a long internal hydrophobic stretch that characterizes such signals (38). Although it does not closely resemble any known mitochondrial signal sequences, it might nevertheless serve as a targeting signal for the mitochondrial matrix since these appear to be quite variable (10). Therefore, even if there is only a single SCP-2 gene, alternative combinations of specific posttranslational processing of a single protein could generate different forms of SCP-2 that could be alternatively targeted to mitochondria or peroxisomes or remain in the cytosol. It is not yet ruled out, however, that there may be more than one SCP-2 gene, each of which encodes an isoform of SCP-2 with different targeting properties.

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