Regulation of sterol carrier protein-2 gene expression in rat liver and small intestine

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Abstract Sterol carrier protein-2 (SCP_2) is a peroxisomal protein most highly expressed in non-steroidogenic tissues such as liver and small intestine. We have examined SCP_2 gene expression during development and after alterations in lipid and bile acid metabolism and compensatory cell growth in the rat. The developmental expression of SCP_2 displayed a biphasic pattern of relative mRNA abundance with a peak at day 19 to 20 of fetal life, reaching adult levels by day 14 and after day 14 in small intestine. In adult rats there was no effect on SCP_2 mRNA abundance, or the relative proportions of the four SCP_2 transcripts after gemfibrozil treatment, 30-fold changes in hepatic cholesteryl ester and triglyceride levels, bile ligation, compensatory hepatic or renal growth. However, immunoblot analysis of tissue homogenates revealed that SCP_2 protein is decreased by 75% in the livers of gemfibrozil-treated animals and increased by 5-fold at 48 h in regenerating liver and in the remaining kidney after unilateral nephrectomy. Taken together these results suggest that SCP_2 gene expression is developmentally regulated and modulated translationally or post-translationally in the adult rat by gemfibrozil and compensatory cell growth.—**Baum, C. L., S. Kansal, and N. O. Davidson.** Regulation of sterol carrier protein-2 gene expression in rat liver and small intestine. *J. Lipid Res.* 1993. **34:** 729–739.

Supplementary key words peroxisomal proteins • gemfibrozil • cholesterol • hepatic regeneration • nonspecific lipid transfer protein • compensatory renal hypertrophy

Cholesterol trafficking is critical for the function of all cells and in particular liver, intestine, and steroidogenic tissues which are distinguished by having multiple sources of cholesterol and cell specific routes for cholesterol metabolism. While much is known about cellular cholesterol synthesis and lipoprotein metabolism there is little known about intracellular cholesterol movement, a process postulated to involve a low molecular weight amphipathic protein called sterol carrier protein-2[2] (SCP_2). This supposition is based on in vitro studies that have shown that SCP_2 facilitates the intermembrane transfer of cholesterol (1–4) and enhances the microsomal conversion of lanosterol to cholesterol (5–7), cholesterol to cholesteryl ester (6), bile acids (8), and pregnenolone (2, 9–12).

Determination of the SCP_2 amino acid sequence (13–15) has facilitated the cloning and sequencing of mouse (16), rat (17–21), and human (22, 23) cDNAs. These cDNAs have, in turn, been used to study aspects of SCP_2 gene expression. The rat SCP_2 cDNA hybridizes to four transcripts (0.8, 1.4, 2.1, and 2.7 kilobases (kb)) on Northern blots of rat liver RNA (19, 21). The 0.8 and 1.4 kb transcripts encode a 15 kDa protein (pre-SCP_2) with a 20-amino acid prepeptide that is post-translationally removed to form mature SCP_2 (24, 25). The 2.1 and 2.7 kb transcripts encode a 58 kDa protein referred to as SCPx[2] (21). Billheimer et al. (18) studied SCP_2 gene expression in rat ovaries and found that SCP_2 mRNA abundance was up-regulated in parallel with mRNA for the cholesterol side chain cleavage enzyme in response to a steroidogenic stimulus. Further evidence in support of an in situ role in sterol metabolism comes from a recent study that found that co-expression of the human SCP_2 cDNA with the cDNAs for the side chain cleavage enzyme and adrenodoxin enhanced steroid hormone synthesis in transfected COS-7 cells (22).

Much less is known about the regulation of SCP_2 gene expression or the function of this protein in nonsteroidogenic tissues. Cell fractionation (26) and immunoelectron microscopy (27–30) studies have demonstrated that SCP_2 immunoreactivity is predominantly localized to peroxisomes of rat liver, a process that is thought to be mediated by a C-terminal consensus peroxisomal targeting sequence (15, 19–21). A potential role in peroxisomal lipid metabolism is supported by the finding that rats treated with the peroxisomal proliferator clofibrate have decreased

Abbreviations: SCP_2, sterol carrier protein-2; FABP, fatty acid-binding protein.

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[2]The nomenclature used in this communication follows that recently detailed by Seedorf and Assmann (21). These workers have demonstrated the presence of two major translation products from a single SCP_2 gene. These products will be referred to as SCP_2, a ~14 kDa protein of 143 amino acids, which is identical to nonspecific lipid transfer protein, and SCPx, a 58 kDa protein of 547 amino acids, which is collinear in its carboxyl terminus with SCP_2.

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hepatic levels of translatable SCP_2 mRNA and protein, while SCPx protein and mRNA are increased (19, 25).

In order to further characterize the regulation of SCP_2 gene expression, we have examined the effects of development, cell growth, and alterations in hepatic and intestinal lipid metabolism. The results demonstrate that SCP_2 gene expression is regulated during development and that SCP_2, but not SCPx, is regulated translationally or post-translationally in the adult rat by the peroxisomal proliferator gemfibrozil and in response to cell growth. However, massive changes in cellular lipid and bile acid metabolism had no effect on SCP_2 gene expression which appears to be constitutive in the small intestine and liver of the adult rat.

MATERIALS AND METHODS

Animals and treatment protocols

Unless otherwise stated, male Sprague-Dawley rats weighing 200–250 g were obtained from Charles River, Wilmington, MA and used for all experiments. Animals were housed, four per cage, in temperature- (20°C) and light- (on between 6 AM and 6 PM) controlled rooms and fed Purina rat chow (Ralston-Purina, St. Louis, MO) ad libitum for at least 10 days prior to entry into the study protocol. Groups of rats as described underwent the following surgical and dietary treatments.

Experiment 1: Development. Timed-pregnant, Sprague-Dawley rats were obtained at 10 days post coitum. Fetuses were removed at 15, 17, 18, 19, 20, and 21 days of gestation. Newborn (day 1), suckling (day 8), and rats aged 14 days and 3 months (adult) were also used. Livers and intestines were removed and snap-frozen in liquid nitrogen and stored at −80°C until they were used for RNA preparation.

Experiment 2: Effects of cholesterol feeding, gemfibrozil treatment, and alterations in hepatic lipogenesis. Rats were divided into five groups, each consisting of six animals. The control animals were fed the standard Purina rat chow ad lib. Two test groups were fed the control diet to which either 2% cholesterol or 0.2% gemfibrozil was added. Cholesterol feeding was performed for 2 weeks, a period of time previously demonstrated to increase hepatic cholesteryl ester levels (31) and gemfibrozil feeding for 1 week (32) which resulted in peroxisomal proliferation confirmed by electron microscopy. The other group of animals was fasted for 48 h or fasted for 48 h and refed a fat-free high carbohydrate diet for 48 h as previously described (33). At the end of the experiments, animals were killed and liver and intestinal mucosal scrapings were harvested and snap-frozen in liquid nitrogen.

Experiment 3: Effect of bile ligation. Rats were anesthetized with ether and subjected to laparotomy alone (sham-operated controls) or bile duct ligation. After 4 days all animals were killed and the livers were removed and snap-frozen in liquid nitrogen.

Experiment 4: Effects of partial hepatectomy or unilateral nephrectomy. Rats were anesthetized with ether and subjected to laparotomy alone (sham-operated controls) or to 70% partial hepatectomy (34) or unilateral nephrectomy. Animals treated with 70% hepatectomy were fed 10% sucrose in their drinking water for the first 24 h post-operatively. At 24, 48, and 120 h after surgery animals were killed and the liver remnant or remaining kidney was removed and snap-frozen. The liver and kidneys were removed from sham-operated animals after the same time intervals.

Tissue levels of SCP_2 by immunoblotting

Frozen rat tissues were homogenized on ice for 1 min at a speed setting of 5 with a Brinkman Polytron in the following homogenization buffer/protease inhibitor cocktail: 50 mM mannitol, 10 mM Tris (pH 7.4), 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 25 μM N-*p*-tosyl-L-lysine chloromethyl ketone, 100 μM leupeptin, 5 mM EDTA, 450 μM aprotinin, and 2 μM pepstatin. The protein concentration of homogenates was measured by the method of Bradford (35) and aliquots were stored at −80°C for subsequent lipid extraction.

Protein samples were separated by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (36) and subsequently electroblotted (33) to polyvinylidene difluoride membranes (Immobilon, Millipore, Bedford, MA). Primary antibody was detected either by ^{125}I-labeled protein A as previously described (33) or with an anti-rabbit horseradish peroxidase-labeled secondary antibody and the ECL chemiluminescence kit according to the company's directions (Amersham, UK). Relative protein abundance was determined by quantitative laser scanning densitometry.

Preparation and characterization of anti-SCP_2 antisera

A polyclonal rabbit anti-rat SCP_2 antiserum was generated after immunization with the homogenous 14 kDa SCP_2 protein prepared from rat liver (1). The specificity of this antiserum was determined by comparison to immunoblots of rat tissues analyzed using immunoaffinity-purified anti-rat SCP_2 IgG (12) provided by Dr. Terrence Scallen (University of New Mexico, Albuquerque, NM). As further validation of the antiserum used in these studies, antibody elution was undertaken from either blotted, purified SCP_2 or from the 58 kDa protein band (SCPx) identified in blotted homogenates of rat liver, as previously described (37).

RNA extraction and analysis of mRNA abundance

Total tissue RNA was extracted into 5 M guanidine thiocyanate as described (38). All preparations of RNA

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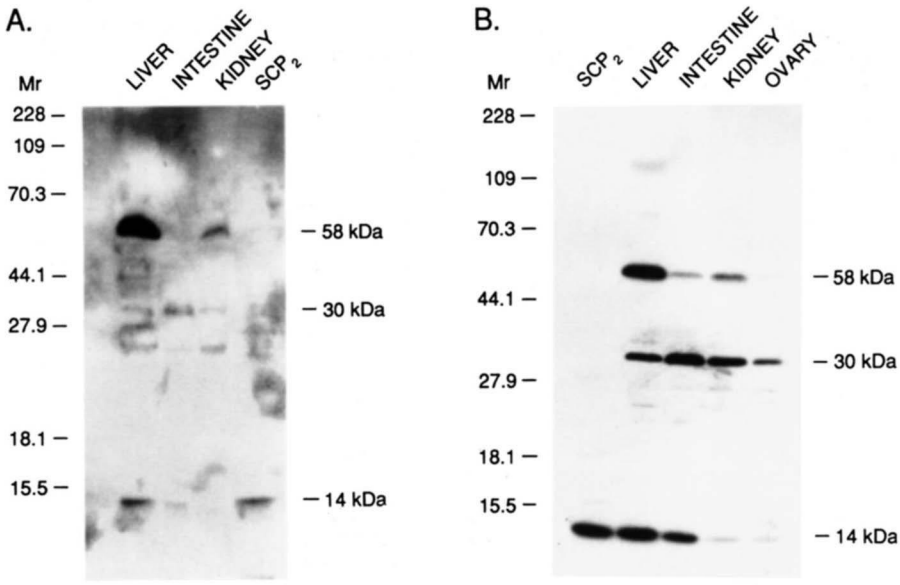

Fig. 1. Immunochemical specificity of anti-SCP_2 antisera. Rat liver, small intestinal mucosa, kidney, and ovary were homogenized on ice in a buffered protease cocktail (Materials and Methods). Fifty-μg aliquots of tissue homogenates were electrophoresed through 12.5% SDS-PAGE gels and transferred to PVDF membranes. The blots were immunostained with affinity-purified anti-rat SCP_2 antiserum followed by detection with an anti-rabbit horseradish peroxidase-labeled secondary antibody-catalyzed chemiluminescence reaction (Materials and Methods). A: Immunoblot of rat liver, small intestine, and kidney probed with an immunoaffinity-purified antibody prepared by elution from blotted purified SCP_2. The migration of SCP_2 was confirmed by the co-electrophoresis of purified SCP_2 (lane 4). B: Immunoblot of rat liver, small intestine, kidney, and ovary probed with an immunoaffinity-purified anti-SCP_2 IgG (12). The migration of SCP_2 was confirmed by the co-electrophoresis of purified SCP_2 (lane 1).

were determined to be intact as judged by analytical 1% agarose-formaldehyde gel electrophoresis of duplicate 20-μg aliquots. Northern blots were prepared using nitrocellulose membranes and were hybridized to various cDNAs as previously described (33). cDNA probes for rat apoA-I (39) and L-FABP (40) were provided by Dr. Jeffrey Gordon (Washington University, St. Louis, MO). cDNA for SCP_2 (18) was provided by Dr. Jeffrey Billheimer (E. I. DuPont de Nemours & Co. Inc., Wilmington, DE). cDNA for 18 S ribosomal RNA (41) was provided by Dr. Ramareddy Guntaka (University of Missouri, Columbia, MO). All inserts were labeled by random priming and hybridized as previously described (33). Relative mRNA abundance was determined by quantitative laser scanning densitometry and values were normalized to 18 S RNA.

Lipid analysis

Tissue lipid was extracted from homogenates (42) and triglyceride was separated by thin-layer chromatography using Silica G in a solvent system of hexane–ethyl ether–acetic acid 70:30:1 (by volume). Triglyceride was recovered from the silica gel by extraction into chloroform–methanol 2:1 (v/v) and fatty acid methyl esters were prepared and analyzed as previously described (33). Hepatic unesterified and esterified cholesterol levels were determined by gas–liquid chromatography as previously described (33).

Statistical methods

All results are expressed as means ± SD. Minitab Statistical Software (State College, PA) was used to perform analysis of variance and Dunnett's test for multiple comparisons.

RESULTS

Immunochemical Specificity of Anti SCP_2 Antisera

Fig. 1 illustrates immunoblots (50 μg/lane) of rat liver, small intestinal mucosa, kidney (and ovary in Fig. 1B), and purified SCP_2 probed with two sources of immunoaffinity-purified anti-SCP_2 antisera. Fig. 1A demonstrates a blot probed with blot immunoaffinity-purified anti-SCP_2 antibody. This antibody detects hepatic proteins of 25, 27, 30, 44, and 120 kDa (partially obscured by background in panel A but more clearly seen in panel B) in addition to SCP_2 (14 kDa) and SCPx (58 kDa). The 30 kDa protein and SCP_2 are the major isoforms in intestine and the 25, 30, and SCPx proteins are predominant in the kidney (small amounts of SCP_2 immunoreactivity are detected on longer exposures). A similar immunoblot of purified SCP_2, rat liver, intestine, and kidney homogenate was obtained with antibody immunoaffinity-purified from the 58 kDa SCPx protein (data not shown). A blot of purified SCP_2, rat liver, small intestinal mucosa, kidney, and ovary homogenates probed with im-

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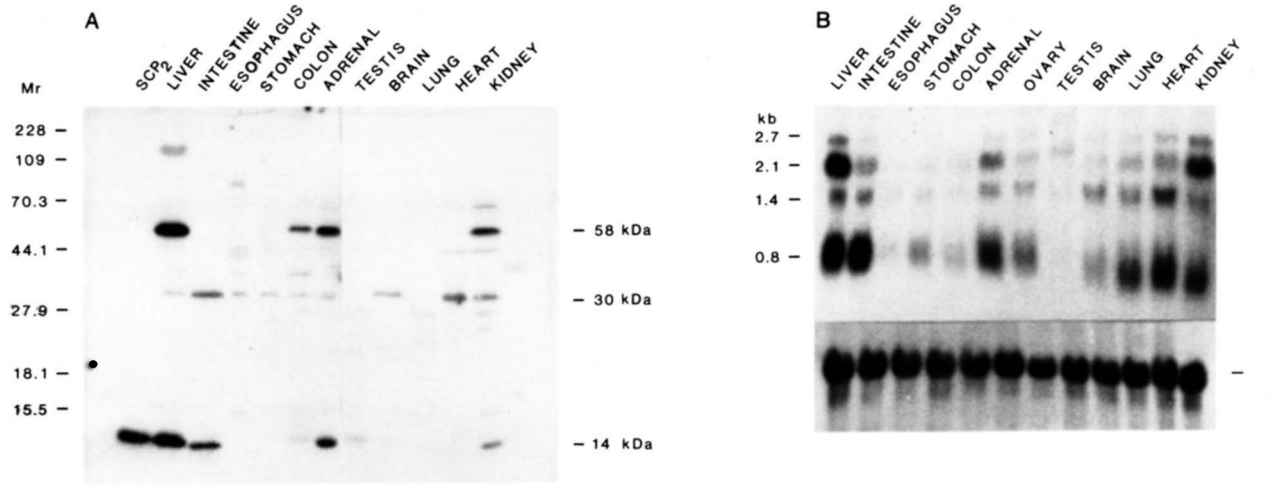

Fig. 2. Tissue-specific SCP_2 gene expression. A: Rat tissues were homogenized in a buffered protease cocktail (Materials and Methods). Fifty-μg aliquots of each tissue homogenate were electrophoresed through 12.5% SDS-PAGE slab gels and transferred to PVDF membranes. The membranes were immunostained with anti-rat SCP_2 antiserum followed by ^{125}I-labeled protein A (Materials and Methods). The migration of SCP_2 was confirmed by the co-electrophoresis of purified SCP_2. B: Total tissue RNA was extracted and aliquots (20 μg) were electrophoresed through a formaldehyde, 1% agarose gel and transferred to nitrocellulose. The blot was probed with cDNAs to SCP_2 and 18 S ribosomal RNA (Materials and Methods).

munoaffinity-purified IgG (12) is shown in Fig. 1B. This predominantly demonstrates proteins in liver of 120, 58 (SCPx), 30, and 14 kDa (SCP_2) and the 30 kDa protein, SCPx, and SCP_2 in intestine and kidney. Homogenates of ovary contain the 30 kDa protein and low levels of SCP_2 and no detectable SCPx. On longer exposures, it is possible to clearly discern protein isoforms in the range of 25–45 kDa. As these affinity-purified antibodies demonstrated the same specificity for purified SCP_2 and SCPx in tissue samples as the unfractionated polyclonal antisera (see Fig. 2A) all of the following studies were performed without prior immunoaffinity purification.

Tissue-specific SCP_2 gene expression in the rat

Protein distribution. **Fig. 2A** illustrates the relative levels of SCP_2 and SCPx protein in homogenates of rat tissues (50 μg/lane) determined by immunoblotting with polyclonal anti-SCP_2. SCP_2 was found in highest abundance in liver followed by small intestinal mucosa, adrenal, and kidney. After longer exposures, SCP_2 was detectable in all tissues as was the 30 kDa protein. The 58 kDa protein, SCPx, was prominent in liver, colon, adrenal, and kidney and found in low abundance in small intestinal mucosa (see Fig. 1B).

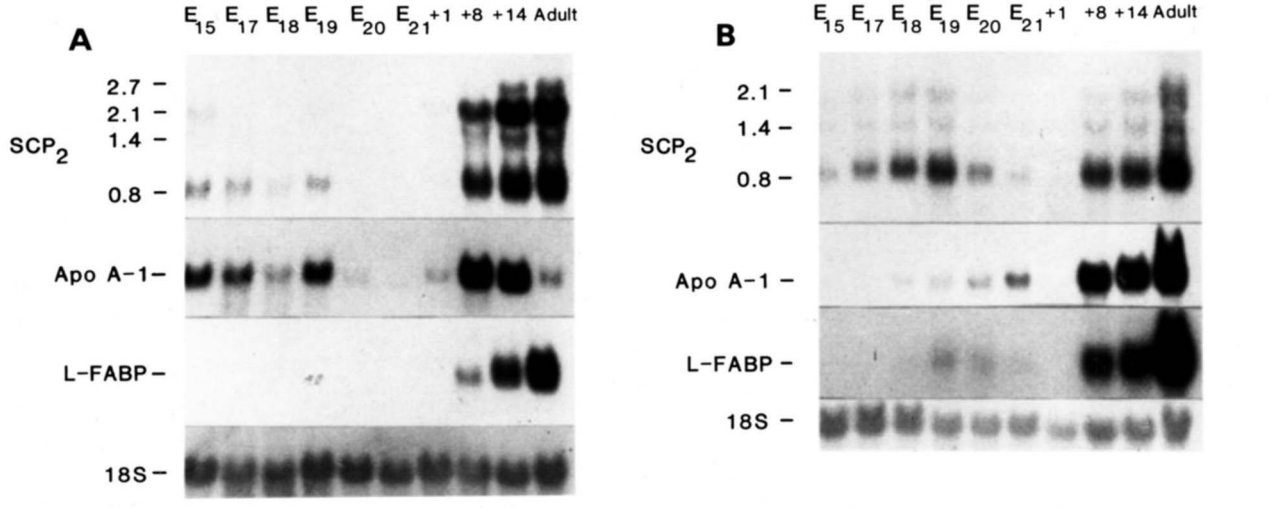

Fig. 3. SCP_2, apoA-I, and L-FABP gene expression: differential regulation during hepatic and small intestinal development. Total RNA was extracted from fetal (E15, E17, E18, E19, E20, E21), neonatal (+1, +8, +14), and adult rat liver and small intestine. Twenty-μg aliquots were electrophoresed through formaldehyde, 1% agarose gels, and transferred to nitrocellulose membranes. Blots were probed with cDNAs to SCP_2, apoA-I, L-FABP, and 18 S ribosomal RNA (Materials and Methods). A: Developmental regulation of SCP_2, apoA-I, and L-FABP mRNA abundance in rat liver. B: Developmental regulation of SCP_2, apoA-I, and L-FABP mRNA abundance in rat small intestine.

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TABLE 1. Hepatic lipid concentrations: modulation by cholesterol and gemfibrozil feeding, fasting and refeeding a high carbohydrate diet, and bile ligation

Experimental Group	No.	TG	FC	CE
			μg/mg protein	
1. Control	6	1.4 ± 0.9	7.7 ± 0.9	1.8 ± 1.4
2. 2% Cholesterol	6	12.0 ± 6.5[a]	9.1 ± 4.8	27.0 ± 1.5[b]
3. Gemfibrozil	6	2.8 ± 0.1	7.8 ± 1.6	0.9 ± 0.1
4. 48 h-Fasted[c]	6	0.8 ± 0.4	10.9 ± 4.5	2.1 ± 1.7
5. 48-h Fasted/48-h refed[c]	6	25.5 ± 5.8[b]	12.8 ± 5.4	5.7 ± 2.6[a]
6. Bile ligated	3	8.1 ± 1.2[a]	8.0 ± 3.0	0.7 ± 0.1

Groups of animals (no./group) were fed a regular chow diet ad libitum for 10 days prior entry into the study protocols. Control animals (group 1) consumed regular chow diet ad libitum until study. Groups 2 and 3 received the control diet to which either 2% cholesterol or 0.2% gemfibrozil, respectively, was added. Cholesterol feeding (group 2) was performed for 1 week and gemfibrozil feeding (group 3) for 2 weeks. Group 4 (48-h fasted) was studied after 48 h of fasting. Group 5 (48-h fasted/48-h refed) was fasted for 48 h and refed a high carbohydrate diet for 48 h prior to study. Group 6 animals (bile ligated) were subjected to bile duct ligation for 4 days. Hepatic lipid concentrations of sham-operated controls (killed 4 days after laparotomy) were within 5% of control values (results not shown).

[a] Signifies $P < 0.01$ compared with control ad libitum-fed using one-way ANOVA and Dunnett's test.
[b] Signifies $P < 0.001$ compared with control ad libitum-fed using one-way ANOVA and Dunnett's test.
[c] Data from reference 33.

RNA distribution. Northern blot analysis of total RNA prepared from the same panel of tissues presented in Fig. 2A revealed that the 0.8 and 1.4 kb transcripts that encode SCP_2 (21) were detected in all tissues analyzed (Fig. 2B). The pattern of 0.8 kb transcript expression is qualitatively similar to the levels of SCP_2 protein in liver, intestine, adrenal, and kidney. Exceptions to this observation were the heart and lung, which expressed high levels of the 1.4

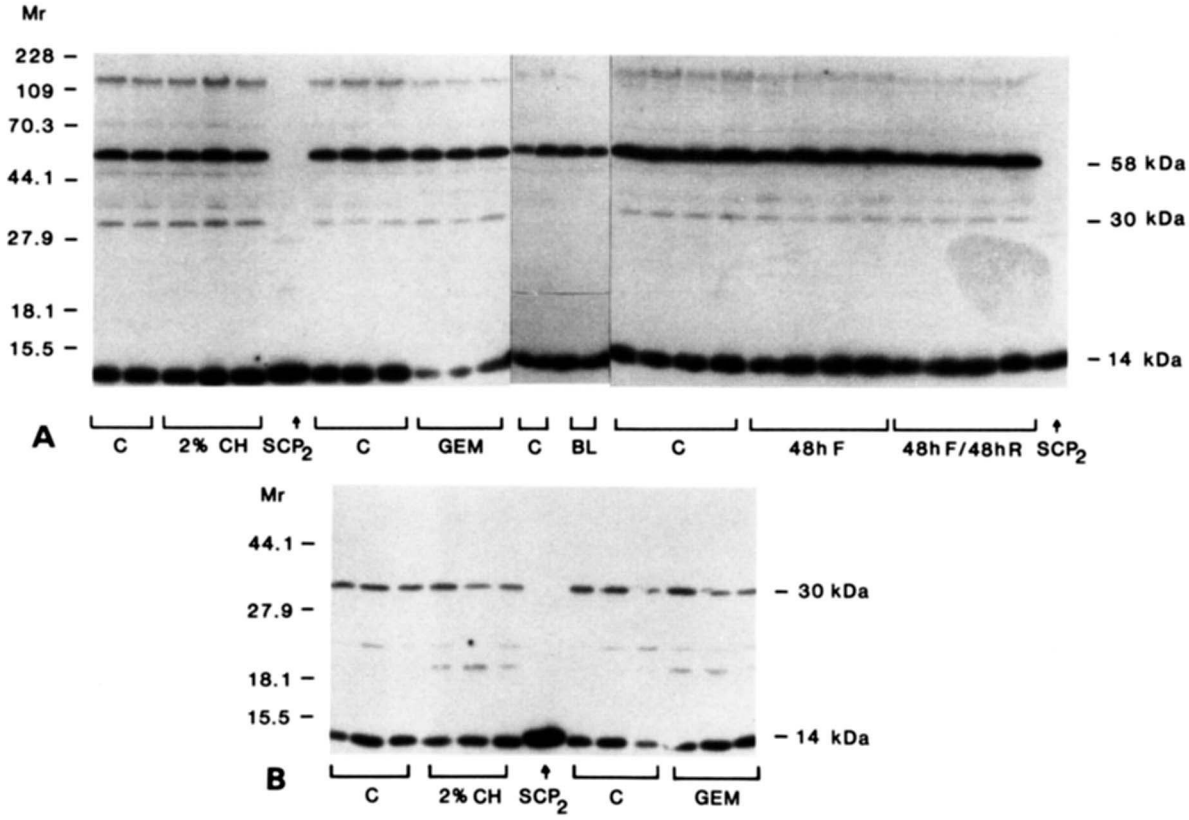

Fig. 4. Immunoblot analysis of hepatic and small intestinal SCP_2, and immunocrossreactive isoforms: effect of alterations in lipid and bile acid metabolism. Replicate aliquots (50 μg) of hepatic and intestinal homogenates from animals in each experimental group were electrophoresed through 12.5% SDS-PAGE gels and transferred to PVDF membranes. The membranes were immunostained with anti-rat SCP_2 antiserum followed by ^{125}I-labeled protein A (Materials and Methods). The migration of SCP_2 was confirmed by the co-electrophoresis of purified SCP_2. A: Regulation of SCP_2 protein in rat liver from control, 2% cholesterol-fed (2% CH), 0.2% gemfibrozil-fed (GEM), 4 day bile-ligated (BL), 48 h-fasted (48 h F), and animals fasted for 48 h and refed a high carbohydrate diet for 48 h (48h F/48h R). B: Regulation of SCP_2 protein in rat small intestine from control, 2% cholesterol-fed (2% cH), and gemfibrozil-fed (GEM) animals.

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and 0.8 kb transcripts but low levels of SCP_2 protein (Fig. 2A). The 2.1 and 2.7 kb transcripts, which encode SCPx, were found in highest abundance in tissues that had the highest levels of SCPx, i.e., liver, adrenal, and kidney (Fig. 2A).

Developmental regulation of hepatic and intestinal SCP_2 gene expression

Total RNA prepared from fetal (E_{15}, E_{17}, E_{18}, E_{19}, E_{20}, E_{21}), neonatal (+1, +8, +14), and adult rat liver and small intestine was examined by Northern blotting to determine the developmental profile of transcript abundance (**Fig. 3**). The predominant transcript during prenatal hepatic and intestinal development is the 0.8 kb SCP_2 transcript which is detected at day E_{15}, the earliest time point examined, and increases to a peak at day E_{19}. At day E_{20} in the liver and day E_{21} in the small intestine the levels of this transcript fall to virtually undetectable levels. During the neonatal period the levels of all four transcripts in the liver rise and reach adult levels by day 14 and, in a similar time course, the 0.8, 1.4, and 2.1 kb transcripts rise to adult levels in the small intestine. In both tissues, the relative proportions of the major SCP_2 transcripts remain constant at all developmental time points examined. By contrast, the developmental expression of apoA-I and L-FABP, two abundant mRNA species encoding intestinal and hepatic proteins involved in lipid transport, is divergent from SCP_2 in a tissue-specific pattern. Hepatic apoA-I mRNA abundance is maximal during the neonatal period and declines during adult life. By contrast, intestinal apoA-I mRNA abundance increases to its peak in adult life. L-FABP mRNA expression is predominantly expressed in neonatal and adult liver and intestine, findings similar to previous descriptions (43, 44).

Hepatic and intestinal SCP_2 gene expression: effect of alterations in lipid metabolism

Hepatic and intestinal lipid flux was modulated by feeding 2% cholesterol, 0.2% gemfibrozil, fasting and refeeding-induced lipogenesis, and bile ligation for 4 days, a treatment known to enhance cholesterol and bile acid synthesis (45, 46). Animals fed a 2% cholesterol diet had a 30-fold increase in hepatic cholesteryl ester concentration compared to animals fed 0.2% gemfibrozil (see **Table 1**). Animals fasted for 48 h and refed a high carbohydrate diet for 48 h have been previously noted to have a greater than 30-fold increase in hepatic triglyceride levels compared to fasted animals (33). There was no effect of cholesterol or gemfibrozil feeding on intestinal triglyceride, cholesteryl ester, and unesterified cholesterol levels (data not shown).

Modulation of SCP_2 protein levels

To determine the effects of these alterations on SCP_2 and SCPx protein levels, samples of rat liver and small intestine were immunoblotted with anti-SCP_2 antisera. SCP_2 and SCPx protein levels in liver were unchanged by cholesterol feeding, bile ligation, and starvation and refeeding-induced lipogenesis (**Fig. 4A**). Gemfibrozil feeding resulted in a 75% reduction in hepatic SCP_2 levels, without an effect on SCPx levels, and a 40% reduction in the level of the 120 kDa protein (Fig. 4A). There was no effect of cholesterol or gemfibrozil feeding on the abundance of either SCP_2 or the 30 kDa protein in small intestine; however, both cholesterol and gemfibrozil feeding resulted in the appearance of a 20 kDa protein (Fig. 4B).

Modulation of SCP_2 mRNA levels

SCP_2 transcript levels, sizes, and the proportions of the

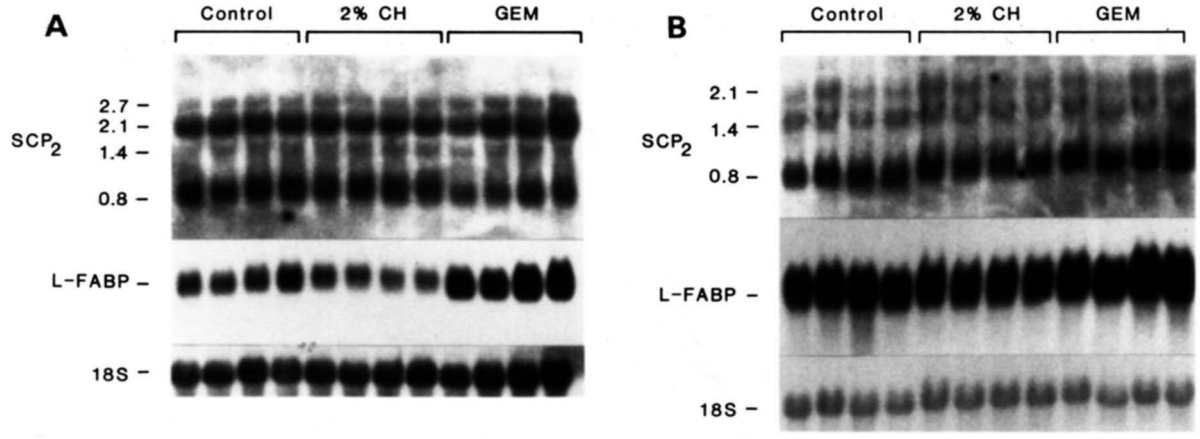

Fig. 5. SCP_2 and L-FABP gene expression: differential modulation by cholesterol and gemfibrozil feeding in rat liver and small intestine. Total RNA was extracted from groups of animals and replicate aliquots (20μg) were electrophoresed through formaldehyde, 1% agarose gels, and transferred to nitrocellulose membranes. Blots were probed with cDNAs to SCP_2, L-FABP, and 18S ribosomal RNA (Materials and Methods). A: Regulation of SCP_2 and L-FABP mRNA abundance in rat liver from control, 2% cholesterol-fed (2% CH), and 0.2% gemfibrozil (GEM)-fed animals. B: Regulation of SCP_2 and L-FABP mRNA abundance in rat small intestine from control, 2% cholesterol-fed (2% CH), and 0.2% gemfibrozil (GEM)-fed animals.

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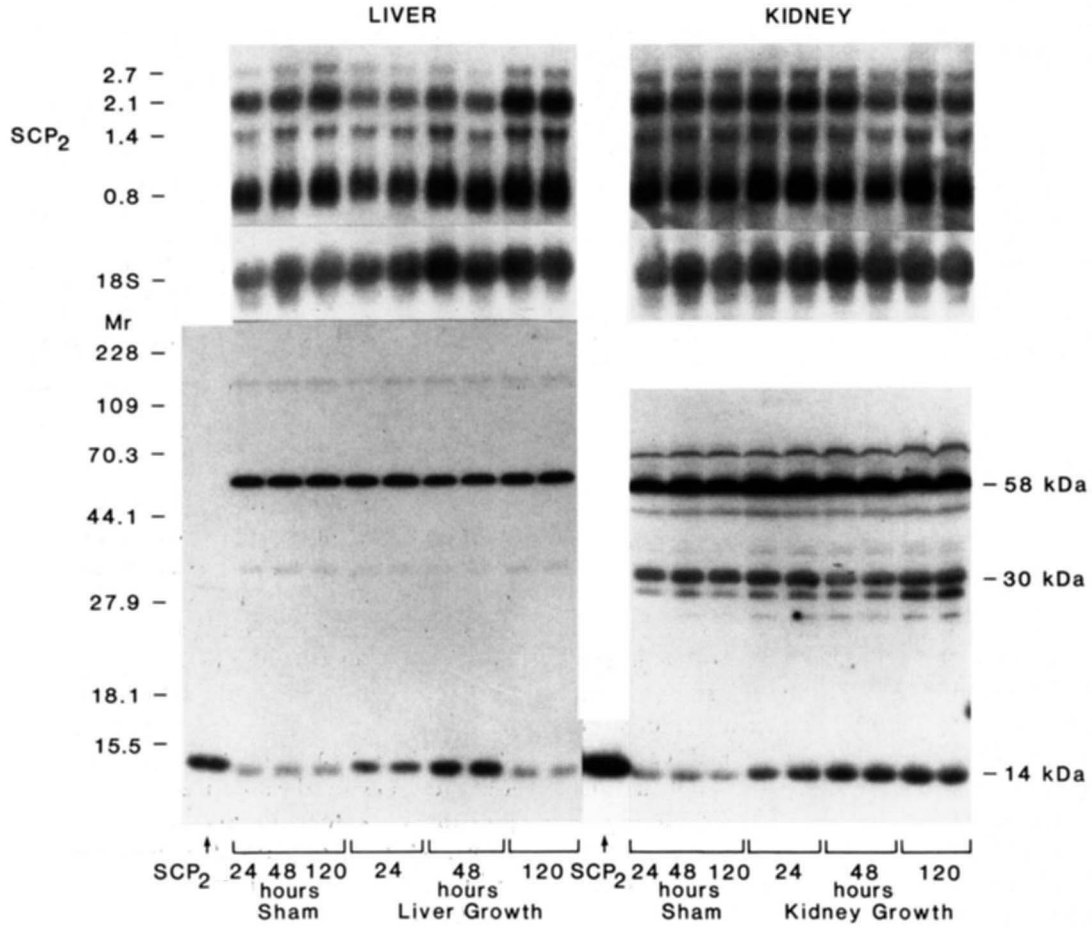

Fig. 6. Regulation of SCP_2 gene expression during hepatic regeneration and compensatory renal hypertrophy. Upper panels: Total hepatic and renal RNA was extracted from sham-operated, regenerating liver (left upper panel) and the remaining kidney after unilateral nephrectomy (right upper panel) at 24 h, 48 h, and 120 h after surgery. Replicate aliquots (20 μg of RNA) were electrophoresed through formaldehyde, 1% agarose gels, and transferred to nitrocellulose membranes. Blots were probed with cDNAs to SCP_2 and 18S ribosomal RNA (Materials and Methods). Lower panels: Hepatic and renal homogenates were prepared from sham-operated, regenerating liver (left lower panel) and the remaining kidney after unilateral nephrectomy (right lower panel) at 24 h, 48 h, and 120 h after surgery. Replicate aliquots (50 μg) of protein homogenate were electrophoresed through 12.5% SDS-PAGE gels and transferred to PVDF membranes. The membranes were immunostained with anti-rat SCP_2 antiserum followed by ^{125}I-labeled protein A (Materials and Methods). The migration of SCP_2 was confirmed by the co-electrophoresis of purified SCP_2.

different transcripts in liver and small intestine were unchanged by 2% cholesterol feeding and gemfibrozil feeding (**Figs. 5A and 5B**) and by bile ligation, and fasting and refeeding-induced lipogenesis (data not shown). By contrast, L-FABP mRNA abundance was regulated in a tissue-specific manner by cholesterol and gemfibrozil feeding (Figs. 5A and 5B). L-FABP RNA levels were decreased 26% by cholesterol feeding and increased 3.5-fold in the liver, but not intestine, of gemfibrozil-fed animals (Fig. 5A).

Hepatic and renal SCP_2 gene expression: modulation by hepatic regeneration and compensatory renal hypertrophy in the adult rat

In order to study the effect of cell growth on SCP_2 and SCPx gene expression, either 70% partial hepatectomy or unilateral nephrectomy was performed on adult rats, and the remaining liver or contralateral kidney, respectively, was removed after 24, 48, and 120 h (**Fig. 6**). Compensatory hepatic or renal growth had no effect on SCP_2 transcript abundance (Fig. 6, upper panels). However, levels of SCP_2 protein increased 3-fold by 24 h and 5-fold by 48 h in both liver and kidney (Fig. 6, lower panels). By 120 h levels of SCP_2 in liver had returned to sham levels, whereas SCP_2 levels in kidney remained elevated. There was no change in SCPx levels in either tissue. In kidney, compensatory growth was also associated with the appearance of 25, 35, and 35.5 kDa proteins, all of which were maximally elevated by 24 h and remained elevated at 120 h. The significance of the 70 kDa band in kidney is not clear as this band was not detected using immunoaffinity-purified anti-SCP_2 antisera (Fig. 1).

The changes in SCP_2 protein during hepatic regeneration temporally paralleled changes in hepatic cholesteryl ester content which increased 5-fold at 24 h and 4-fold at 48 h, and with hepatic triglyceride levels, which increased 2-fold at 48 h (**Table 2**). In a similar manner, compensatory renal hypertrophy resulted in a 20% increase in un-

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TABLE 2. Hepatic and renal lipid concentrations: modulation by compensatory tissue growth[a]

Experimental Group	Hepatic Lipid No.	TG	FC	CE	Renal Lipid No.	TG	FC	CE
		μg/mg protein				*μg/mg protein*		
Sham operated[b]	6	1.1 ± 0.1	7.4 ± 0.8	1.1 ± 0.2	6	5.6 ± 3.4	3.8 ± 0.1	<0.1
Compensatory growth 24 h	3	1.9 ± 0.1	7.3 ± 0.1	6.4 ± 2.8[c]	3	5.2 ± 0.9	4.1 ± 0.9	<0.1
Compensatory growth 48 h	3	2.9 ± 0.1[c]	7.4 ± 0.1	4.8 ± 0.9[c]	3	4.1 ± 0.5	4.5 ± 0.4[c]	<0.1
Compensatory growth 120 h	3	0.7 ± 0.1	7.0 ± 0.7	1.1 ± 0.2	3	2.1 ± 1.2	3.8 ± 0.1	<0.1

[a]Hepatic and renal lipid levels were measured at 24, 48, and 120 h in either the liver remnant after 70% partial hepatectomy or in the remaining kidney after unilateral nephrectomy.
[b]Results from sham-operated animals were pooled because there was less than 10% difference between values at 24, 48, and 120 h.
[c]Signifies $P < 0.01$ compared with sham operated using one-way ANOVA and Dunnett's test.

esterified cholesterol levels, which paralleled increases in the levels of SCP_2 in this tissue.

DISCUSSION

The purpose of these studies was to define factors responsible for modulating SCP_2 gene expression in nonsteroidogenic tissues. The significance of this aim is underscored by the observation that, with the exception of adrenal, SCP_2 is most highly expressed in epithelial tissues, i.e., liver, intestine, and kidney. Of particular importance is an understanding of the regulation and function of SCP_2 in liver and small intestine because these tissues are the main sources of endogenously synthesized cholesterol and serum lipoproteins.

Geelen, Beynen, and Wirtz (47) examined the effect of cholestyramine, cholesterol, mevinolin, cholate, and clofibrate feeding on rat liver cytosolic levels of SCP_2 immunoreactivity measured by enzyme-linked immunoassay. They demonstrated that SCP_2 immunoreactivity decreased 15–25% by the administration of cholate and the hypolipidemic agents mevinolin and clofibrate. The immunocrossreactivity of SCP_2 and SCPx and the potential for other immunoreactive isoforms was not addressed by Geelen et al. (47) and thus the potential for differential regulation of SCP_2 and SCPx could not be determined with the methodology used by these investigators.

We examined SCP_2 gene expression using tissue immunoblot and Northern blot analyses. SCP_2 and SCPx protein and mRNA levels were unaffected by cholesterol feeding, fasting and refeeding-induced lipogenesis, and bile ligation, despite changes in the levels of hepatic cholesteryl ester and triglyceride, and bile acid metabolism. These observations do not discount the possibility that changes in lipid flux regulate the subcellular distribution of SCP_2 with minimal or no effect on total cellular levels, a process that has been demonstrated in rat corpus luteal and Leydig cells after steroidogenic stimulation (48–50).

Other immunologically cross-reactive isoforms of SCP_2 have been identified (3, 24) that may represent post-translationally modified forms of SCP_2 or SCPx or separate gene products; for example, the liver-specific 120 kDa protein or the ubiquitous 30 kDa isoform which is the same size as in vitro translation products encoded by rat SCP_2 cDNA (18). While the significance of these isoforms remains to be determined, the current observations indicate that the 120 kDa hepatic protein is down-regulated in association with gemfibrozil feeding (Fig. 4A). Additionally, the appearance of 25, 35, and 35.5 kDa proteins in kidney in association with compensatory growth (Fig. 6) suggests that there may be a family of "SCP_2-like" proteins with potentially distinct lipid transfer functions and/or alternative subcellular sites of action. Thus, the extraperoxisomal SCP_2 immunoreactivity in rat liver mitochondria and endoplasmic reticulum (28) may represent immunoreactive isoforms of SCP_2 or SCP_2 that has been redirected to different cellular locations. Studies to resolve these issues, using immunoelectron microscopy and anti-peptide antibodies are currently in progress.

While modulation of hepatic lipid and intestinal flux fails to modulate SCP_2 gene expression, hepatic and intestinal development demonstrates a biphasic developmental pattern of SCP_2 mRNA abundance which is similar to the pattern of hepatic and intestinal 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, prenyl transferase, HMG-CoA synthase (51), and apolipoprotein B (apoB) gene expression in the rat (52). The coordinate expression of these genes during fetal life occurs during a developmental period marked by rapid tissue growth and high levels of fetal cholesterol synthesis (51). Furthermore, this developmental pattern is distinct from that of apoA-I and L-FABP, two proteins involved in lipid transport.

The importance of the cholesterol synthetic pathway in cell growth is well recognized as is the requirement for cholesterol for new membrane formation (reviewed in ref. 53). In order to further explore the potential association between cell growth, cholesterol metabolism, and SCP_2 gene expression, we examined compensatory cell growth

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in liver and kidney after partial hepatectomy and in the remaining kidney after unilateral nephrectomy. Levels of SCP_2 mRNA were unchanged while SCP_2 protein increased 5-fold by 48 h, paralleling well-characterized changes in cell growth in both tissues (34, 54). The modulation of SCP_2 levels also parallels previously described changes in hepatocellular cholesterol synthesis during hepatic regeneration (55). An interesting difference between the responses seen in these two tissues is that levels of SCP_2 in the remaining kidney remained elevated at 120 h whereas SCP_2 levels returned to normal by this time in the regenerating liver. This difference is likely to reflect a linear rate of kidney enlargement which persists for at least 120 h but not new cell growth which halts by 72–96 h (54). The rate of new cell growth in liver reaches a peak at 48–72 h in parallel with cholesterol synthesis and declines thereafter (34). Thus the increase in SCP_2 protein in temporal association with cell growth suggests that aspects of growth-specific alterations in cholesterol metabolism may be of importance in understanding the regulation and function of SCP_2.

In contrast to our findings regarding SCP_2, the 2.7 and 2.1 Kb transcripts that encode SCPx were developmentally regulated in parallel with SCP_2 but otherwise constitutively expressed. Tissue levels of SCPx and SCP_2 tended to be coordinately regulated except in the small intestine where levels of SCPx are low (Fig. 2A). This observation is supported by the low abundance of the 2.7 and 2.1 kb transcripts and is consistent with recent studies that have demonstrated the liver-specific expression of other peroxisomal proteins (56). While the significance of this observation remains open, we speculate that SCP_2 function, at least in the small intestine, is not dependent on the presence of SCPx.

In conclusion, SCP_2 gene expression in nonsteroidogenic tissues of the rat appears to be regulated both transcriptionally and post-transcriptionally. The developmental and tissue-specific distribution of SCP_2 involves both the regulation of transcript abundance and the differential modulation of transcripts encoding SCP_2 and SCPx. SCP_2 protein levels are modulated by one or more translational or post-translational processes that appear to function in lung and heart to keep SCP_2 levels low despite relatively high levels of the 0.8 kb transcript. Additionally, SCP_2 protein levels, unlike other peroxisomal proteins and enzymes (56, 57), are divergently regulated by gemfibrozil and compensatory cell growth, both of which are associated with peroxisomal proliferation (32; 58). In contrast, SCP_2 levels parallel effects on cholesterol synthesis induced by the hypolipidemic effects of gemfibrozil and by the increased cholesterol requirements associated with cell growth. These associations support the contention that SCP_2 may function in some aspect of cholesterol metabolism and, in particular, that which occurs in peroxisomes (for review, see ref. 59). Future studies of the regulatory mechanisms that govern SCP_2 levels and subcellular distribution in conjunction with studies designed to elucidate the role of peroxisomes in cellular cholesterol homeostasis will be helpful in determining the cellular function of SCP_2.

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