Sterol Carrier Protein 2 and Fatty Acid-binding Protein

SEPARATE AND DISTINCT PHYSIOLOGICAL FUNCTIONS*

(Received for publication, July 27, 1984)

Terence J. Scallen‡‡, Billie J. Noland‡, Kathleen L. Gavey‡, Nathan M. Bass†, Robert K. Ockner††, Ronald Chanderbhan** and George V. Vahouny‡‡‡

From the ‡Department of Biochemistry and Department of Medicine, School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131, the ¶Department of Medicine, School of Medicine, University of California, San Francisco, California 94143, and the **Department of Biochemistry, The George Washington University School of Medicine and Health Sciences, Washington, D.C. 20037

Sterol carrier protein 2 (SCP-2) participates in the microsomal conversion of lanosterol to cholesterol, in the conversion of cholesterol to cholesterol ester, and in intracellular cholesterol transfers. The stoichiometry of binding between cholesterol and SCP-2 is 1:1. However, reports have appeared attributing sterol carrier protein activity to a protein preparation identical to hepatic fatty acid-binding protein (FABP).

Therefore, the present investigation was conducted to compare homogeneous preparations of FABP and SCP-2 with respect to their capacities to participate as carrier proteins in reactions involving sterols or fatty acids. The results show that SCP-2 and FABP have separate and distinct physiological functions, with SCP-2 participating in reactions involving sterols and FABP participating in reactions involving fatty acid binding and/or transport. Furthermore, there is no overlap in substrate specificities, i.e. FABP does not possess sterol carrier protein activity and SCP-2 does not specifically bind or transport fatty acids.

As long as only small quantities of organic solvent (1.6 volume %) were used for substrate addition, the sterol Δ7-reductase liver microsomal assay for SCP-2 correlated well with the physiologically relevant assays employed in the reconstituted adrenal system. The sterol carrier protein activity previously attributed to rat hepatic FABP is explained by the presence of significant quantities of propylene glycol (15 volume %) or Tween 80 in the assay procedure.

Rat liver cytosol contains two sterol carrier proteins (1). SCP-1 (M, 47,000) participates in the microsomal conversion of squalene to lanosterol (2-4), while SCP-2 (M, 13,500) participates in the microsomal conversion of lanosterol to cholesterol (5). In addition, SCP-2 is required for the transport of cholesterol from cytoplasmic lipid inclusion droplets to mitochondria in the adrenal cortex (6), and it also is capable of translocating cholesterol from the outer to the inner mitochondrial membrane (7). Furthermore, the stoichiometry of binding of cholesterol by SCP-2 is 1:1 (6), and pretreatment of adrenal cytosol with anti-SCP-2 IgG abolishes all of the stimulatory activity of this cytosol on adrenal mitochondrial pregnenolone production (8).

However, reports have appeared attributing sterol carrier protein activity to a protein preparation (9, 10) identical to hepatic FABP (11). There exists, therefore, confusion as to the true nature of hepatic sterol carrier protein. Is it SCP-2 or FABP, or do both proteins participate as sterol carriers? Therefore, the present investigation was conducted to compare homogeneous preparations of FABP and SCP-2 with respect to their capacities to participate as carrier (binding) proteins in reactions involving sterols or fatty acids. The results show that SCP-2 and FABP have separate and distinct physiological functions, with SCP-2 participating in reactions involving sterols and FABP participating in reactions involving fatty acids. There is no overlap in substrate specificities, i.e. FABP does not possess sterol carrier protein activity and SCP-2 does not specifically bind or transport fatty acid. The results of the present study also explain why sterol carrier protein activity was previously attributed to FABP (9).

Preliminary accounts of these experiments have appeared (12, 13).

EXPERIMENTAL PROCEDURES

Materials—Rat liver SCP-2 was purified to homogeneity as previously reported (5). The yield, however, was improved to approximately 30% by the following modifications. (i) During the last step, the 303,000 × g supernatant (SSup) was heated more slowly (18-20 min) to reach a final temperature of 50°C. Heating at 50°C was then continued for 1 min. (ii) A 2-liter Amicon ultrafiltration cell, using a YM-5 membrane, was used in place of the hollow-fiber concentrator to concentrate the SCP-2 fraction obtained from the blue Dextran-Sepharose 4B affinity column. (iii) After concentration and before gel filtration on Sephadex G-75 superfine, the sample was centrifuged at 5000 rpm for 10 min and the precipitate discarded. Using the procedure as previously described (5) and modified as described above, 6 to 7 mg of homogeneous SCP-2 was obtained from the livers of 100 male Sprague-Dawley rats.

Hepatic FABP was purified to homogeneity as described (11). Polyacrylamide Gel Electrophoresis—Polyacrylamide gel electrophoresis was performed as described by Gabriel (14), and gels were stained with Coomassie Brilliant Blue G-250 according to the procedure of Blakesley and Boezi (15). Stacking gels were not used, and gels were subjected to pre-electrophoresis at 2 mA/gel for 3 h.

Assay of SCP-2—SCP-2 activity of rat liver cytosol (303,000 × g supernatant, SSup) and of the homogeneous protein was determined by the stimulation of the conversion of 7-dehydrocholesterol to cholesterol using rat liver microsomes (5), except that the final assay volume was reduced to 0.65 ml.
Briefly, the standard assay procedure is conducted as follows. Incubations contained in a final volume of 0.65 ml: Buffer P (15 mM potassium phosphate buffer (pH 6.80) containing 0.1 mM EDTA), NADPH (1.8 mM), buffer-washed rat liver microsomes (2 mg of protein) (5), 0.1% SCP-2 or SCP-2 or the protein to be evaluated, and 7-dehydrocholesterol (140 μg of 7-dehydrocholesterol microsomes/1 ml assay mixture) ad mixed to achieve a final concentration of 7-dehydrocholesterol was linear over the 2-h incubation period. Control incubations, minus any SCP-2 or SCP-2 or protein addition (microsome blank), or minus SCP-2 or SCP-2 or protein addition and microsomes (substrate blank) were always conducted. No evidence was observed for nonenzymatic oxidation of the 7-dehydrocholesterol substrate. Furthermore, the disappearance of absorbance measured in the ultraviolet at 294 nm corresponded precisely with assays conducted using [14C]-7-dehydrocholesterol substrate in which [14C]cholesterol formation was measured by chromatography on silver nitrate-impregnated silicic acid columns (5). Saponification, extraction, and ultraviolet spectroscopy were conducted as described (5). The value obtained for cholesterol formation for the microsomal blanks was subtracted from the cholesterol formed in incubations containing both microsomes and the protein to be evaluated (5).

For assay conditions previously described in reports (9, 16), which attributed sterol carrier protein activity to FABP, were conducted with respect to the concentration of propylene glycol and preincubation procedures as follows, combining the components listed: Buffer P (213 μl) and propylene glycol (87 μl) mixed together, NADPH in Buffer P (50 μl), the protein sample to be evaluated (100 μl), and 7-dehydrocholesterol. The incubation mixture was preincubated at 37 °C for 15 min. Then microsomes (200 μl) were added, and incubation was performed for 2 h at 37 °C.

Fatty Acid-binding Assay—Fatty acid binding to hepatic FABP or SCP-2 was analyzed by a modification of the assay described by Glatz and Stoffel (8) for studying fatty acid binding of hydrophobic ligands to Lipidex 1000 (Packard Instrument Co.), a substituted hydroxylalkoxypropyl derivative of Sephadex G-25 (17, 18). Thus, free, but not protein-bound fatty acids, partition into Lipidex at 0-4 °C. The assay, as carried out in these studies, was conducted as follows. The protein to be evaluated (10–14 μg) was incubated with [3H]oleate in 1.5 ml propylene glycol in 5 μl potassium phosphate buffer, pH 7.4. Radiolabeled oleic acid, dissolved in 5 μl of propylene glycol, was added to obtain a concentration range of 0.5 to 20 μM. The final assay volume was 0.455 ml. After incubation of protein and oleate for 10 min at 37 °C, the vials were cooled, and 80 μl of ice-cold Lipidex 1000 (1:1, v/v), in 15 mM Tris buffer (pH 7.4), was added to remove unbound fatty acids. After mixing, samples were incubated at 0 °C for 10 min, centrifuged at 20,000×g at 4 °C for 2 min, and the supernatants were assayed for radioactivity in 10 ml of Liquifluor (New England Nuclear) in toluene containing 10% BioSolv (Packard) (20), and then 200 μl of a solution of 0.5% gelatin (Type IV, Sigma) in PBS was added to each well and incubation was conducted for 30 min at 37 °C. The plates were washed again three times as described above. Anti-SCP-2 IgG and preimmune (control) IgG were adjusted to equivalent protein concentrations (1.5 mg/ml), diluted 1:250 using PBS/T, and 200-μl aliquots were added to appropriate wells. After incubation at 37 °C for 30 min, the solutions were aspirated and the wells were washed as described above. Goat anti-rabbit IgG, (200 μl), peroxidase conjugated (Cappel, –1 mg/ml and then diluted 1:800), was added to appropriate wells. The plates were incubated for 30 min at 37 °C, solutions aspirated, and washing conducted as described above. The substrate solution for the peroxidase reaction (20 ml), containing 0.02% (w/v) 2,2'-azinodi-(3-ethylbenzthiazoline) sulfonic acid, (Sigma) in 0.12 M citric acid (adjusted to pH 4 with 5 M KH2PO4) was added at timed intervals and, after 15 min, the reaction was stopped with 50 μl of 154 mM sodium chloride. The value obtained for the incorporation of [4-14C]cholesterol into the lipid droplets (23). The washed mitochondrial pellets were resuspended in pH 7.5 buffer (24) containing 36 mM Tris, pH 7.5, 10.4 mM sodium phosphate, pH 7.5, 13 mM sucinate, 5.2 mM magnesium chloride, and 0.25 mM sucrose. Rat adrenal cytoplasmic lipid inclusion droplets were isolated as described (6) using a 50 mM sodium phosphate buffer, pH 7.4, containing 5 mM MgCl2 and 154 mM sodium chloride. [1-14C]Arachidonate was incorporated into the lipid droplets by injection of 5 μCi in 5 μl of acetone into the suspension of lipid droplets using a Hamilton syringe. The suspension was incubated, and the lipid droplets were reisolated and washed by ultracentrifugation as described for the incorporation of [4-14C]cholesterol into the lipid droplets (6).

RESULTS AND DISCUSSION

Characterization of SCP-2 and FABP—A comparison of the electrophoretic mobility of purified SCP-2 and rat liver FABP at pH 4.3 was performed (Fig. 1). As can be seen, SCP-2 migrates more rapidly toward the negative electrode than does FABP. This is consistent with the pl for SCP-2 which is ~8.6 (5), while the pl for FABP is 6.9 (11). It is also apparent (Fig. 1) that both SCP-2 and rat liver FABP are essentially homogeneous.

The amino acid compositions of homogeneous SCP-2, FABP (11), and the protein preparation reported to have SCP activity (9) are shown in Table I. As can be seen, there are marked differences in the amino acid composition of SCP-2.
and either of the other proteins, particularly in the quantities of threonine, alanine, valine, and leucine. In addition while tyrosine is present in FABP and the protein preparation reported to have SCP activity (9), it is not found in SCP-2. The correlation coefficient between SCP-2 and the protein with reported SCP activity (9) is 0.736. Clearly, SCP-2 and this protein are separate and distinct proteins.

On the other hand, the amino acid compositions of purified hepatic FABP and the protein preparation of Dempsey et al. (9) (Table I) are virtually identical, with a correlation coefficient between the two protein preparations being 0.997.

Homogeneous SCP-2 and FABP were compared for immunological cross-reactivity against anti-SCP-2 IgG (Table II). There was no immunological cross-reactivity between SCP-2 and FABP, using an extremely sensitive enzyme-linked immunosorbent assay procedure. Also, it was found that anti-rat liver FABP antiserum did not react with pure rat liver SCP-2 on radial immunodiffusion.

FABP and SCP-2 were then evaluated by means of the following six assay procedures.

Cholesterol Release from Adrenal Cytoplasmic Lipid Inclusion Droplets—The release of cholesterol from adrenal cytoplasmic lipid inclusion droplets is an essential step in the process of steroidogenesis in the adrenal. Therefore, we examined the capacity of SCP-2 or FABP to release cholesterol mass from these cytoplasmic lipid inclusion droplets as a function of SCP-2 or FABP concentration. With SCP-2, but not with FABP, there was a concentration-dependent release of cholesterol mass from the cytoplasmic lipid inclusion droplets (Fig. 2A).

Pregnenolone Production by Adrenal Mitochondria—The synthesis of pregnenolone from endogenous cholesterol was measured as a function of either SCP-2 or FABP concentration. This assay procedure mirrors the final step in adrenal steroidogenesis, i.e. the production of the first adrenal steroid hormone from endogenous mitochondrial cholesterol. With SCP-2, but not with FABP, there was a concentration-dependent formation of pregnenolone by the adrenal mitochondria (Fig. 2B).

Arachidonate Release from Adrenal Cytoplasmic Lipid Inclusion DROPLETS—The release of [1-14C]arachidonate from cytoplasmic lipid inclusion droplets was measured for FABP and SCP-2 (Table III). With FABP, but not with SCP-2, there was a substantial increase in the release of [1-14C]arachidonate from the cytoplasmic lipid inclusion droplets.

Oleic Acid Binding—The binding of oleic acid was measured for both proteins (Fig. 3). With FABP, but not with SCP-2, there was specific and saturable binding of oleic acid (Kd = 1.5 ± 0.22 μM) involving a single binding site/molecule and a minor nonsaturable process with a slope of 0.025. Binding of the fatty acid to SCP-2 was fitted best by a linear nonsaturable process (slope = 0.12), possibly explained by nonspecific association of fatty acid aggregates with SCP-2.

Cholesterol Esterification—Rat liver acyl-CoA cholesterol esterification
acyl transferase activity was evaluated in the presence of SCP-2 or FABP. With [4-14C]cholesterol as substrate, SCP-2 produced a marked enhancement of acyl-CoA cholesterol acyl transferase activity (Fig. 4A), when compared with microsomes with no protein added or when FABP was added. A similar experiment (data not shown), using unlabeled cholesterol and labeled palmitoyl-CoA, also showed marked enhancement of cholesterol palmitate formation by SCP-2, but FABP (1.06 μM) was without effect.

The results of studies in which acyl-CoA cholesterol acyl transferase activity was measured in the presence of FABP, but at much higher concentrations, are shown in Fig. 4B. Using concentrations of FABP which were 10- to 30-fold higher than in Fig. 4A, FABP produced a modest enhancement of acyl-CoA cholesterol acyl transferase activity using labeled palmitoyl-CoA as substrate. The effective concentration of FABP in this instance is similar to that shown previously by Burnett et al. (28) for other reactions involving fatty acyl-CoA compounds as substrates.

Effect of Assay Conditions on Sterol Δ⁷-Reductase—The question remained as to why sterol carrier protein activity has been attributed to a preparation of hepatic FABP (9). This question was investigated by comparing the assay conditions employed in the in vitro assay (9, 16), which contains approximately 15 volume % organic solvent (propylene glycol) used for substrate addition, with the procedure described (6) which uses small quantities (1.6 volume %) of organic solvent for substrate introduction.
Comparison of SCP-2 and FABP; Separate Physiological Roles

The results of studies in which the liver microsomal conversion of 7-dehydrocholesterol to cholesterol (sterol Δ7-reductase) was measured with varying amounts of SCP-2 or FABP are shown in Fig. 5. In the experiment shown in Fig. 5A, the assay procedure (5), which uses a small quantity of organic solvent (dioxanepropylene glycol, 2:1, 1.6 volume % in the incubation) for substrate addition, showed marked activity for purified SCP-2 in stimulating the conversion of 7-dehydrocholesterol to cholesterol by rat liver microsomes. When the assay conditions of Dempsey, (9, 16) are employed (preincubation for 15 min at 37 °C with the protein addition and with approximately 15 volume % of organic solvent (propylene glycol) present), the activity of purified SCP-2 was markedly inhibited. When a similar experiment was conducted with FABP (Fig. 5B), no sterol carrier protein activity was observed as long as only small amounts of organic solvent (1.6 volume %) were used for substrate addition (5) (see “Experimental Procedures”). However, when approximately 15 volume % organic solvent (propylene glycol) was present in the incubations as in (9, 16, 29), small amounts of SCP activity were detected with FABP, but only at levels of protein far greater than for SCP-2.

A similar phenomenon was observed with bovine serum albumin (Table IV), i.e. no SCP activity was observed when some liposome exchange system. But since a liposome is an organic inclusion of droplets or when the propylene glycol similar to Dempsey et al. (9, 16). The protein addition in each flask was bovine serum albumin (4 mg/assay). Assays were conducted as described under “Experimental Procedures.”

small amounts of organic solvent (1.6 volume % in the incubations) were used for substrate introduction; however, SCP-like activity was observed when bovine serum albumin (4 mg) was preincubated with increasingly amounts of propylene glycol prior to the addition of microsomes. With no preincubation, the effect of the propylene glycol was somewhat less.

The reconstituted adrenal assays employed in the present investigation, i.e. cholesterol mass release from adrenal cytoplasmic lipid inclusions droplets or pregnenolone production from endogenous cholesterol present in adrenal mitochondria, mirror precisely the physiologically important process of steroidogenesis in the adrenal and other endocrine tissues. These assays do not require the use of organic solvents for substrate addition. The substrate cholesterol is presented in physiological donors, i.e. cytoplasmic lipid inclusion droplets or mitochondria. Both of these organelles are known to be involved, in vivo, in the biosynthesis of pregnenolone from cholesterol. Since it is shown in the present investigation that FABP is not capable of releasing cholesterol mass from adrenal lipid droplets (Fig. 2A) or stimulating the utilization of endogenous mitochondrial cholesterol for pregnenolone production (Fig. 2B), it is clear that FABP is not involved as a sterol carrier protein in a physiological sense. Consistent with this finding is the observation of Mishkin et al. (30) that FABP does not bind sterol. It is also clear that the physiologically relevant assays employed in the reconstituted adrenal system (Fig. 2) correlate well with the rat liver microsomal sterol Δ7-reductase assay (Fig. 5), in which small quantities (1.6 volume % in the incubations) of organic solvent are used for substrate addition. However, it is apparent that significant difficulties occur when larger quantities of organic solvent (propylene glycol) are used in the assay procedure (Fig. 5 and Table IV) and as described (9, 16).

Problems similar to those observed with propylene glycol (Fig. 5 and Table IV) were also observed when Tween 80 (0.05%) was present during the assay (Table V), i.e. bovine serum albumin shows SCP-like activity in the presence of detergent. Therefore, it is not desirable to use detergent as a substrate vehicle as described (31) for studies utilizing a preparation of hepatic FABP.

Previously (5), it was shown that the amino acid composition of a protein described as a nonspecific phospholipid exchange protein (CM2) (32) was virtually identical to SCP-2. The correlation coefficient between SCP-2 and CM2 is equal to 0.992 (5). We have shown that SCP-2 has in vitro phospholipid exchange activity as assayed by using a microsome/ liposome exchange system. But since a liposome is an

| Propylene glycol added to assay | Cholesterol formed |
|-------------------------------|--------------------|
|                               | Preincubation | No preincubation |
| µl | nmoles/2 h | µl | nmoles/2 h | µl | nmoles/2 h |
| 0  | 0          | 0  | 0          | 0  | 0          |
| 10 | 0          | 0  | 10         | 0  | 10         |
| 20 | 0          | 0  | 16         | 50 | 3.0        |
| 50 | 12.0       | 13.5| 34         | 125| 12.0       |
| 34.5| 55         | 125| 12.0       | 125| 12.0       |
| 17 | 0          | 0  | 200        |

A | B

FIG. 5. The conversion of 7-dehydrocholesterol to cholesterol by rat liver microsomes is compared using two assay procedures. The assay procedure which uses a small amount (1.6 volume %) of organic solvent (Org. Sol.) for substrate introduction is compared with the assay (9, 16) which contains approximately 15 volume % organic solvent (propylene glycol). See “Experimental Procedures” for additional details. Each point represents the mean of duplicate determinations. A, the two assay procedures are compared for SCP-2; B, the two assay procedures are compared for FABP.

The effect of adding propylene glycol in the presence of bovine serum albumin on the microsomal conversion of 7-dehydrocholesterol to cholesterol

| Amount of propylene glycol added to assay | Cholesterol formed |
|------------------------------------------|--------------------|
|                                          | Preincubation | No preincubation |
|                                          | µl | nmoles/2 h | µl | nmoles/2 h | µl | nmoles/2 h |
| 0  | 0          | 0  | 0          | 0  | 0          | 0  | 0          |
| 10 | 0          | 0  | 10         | 0  | 10         | 0  | 10         |
| 20 | 0          | 0  | 16         | 50 | 3.0        | 34 | 28         |
| 50 | 12.0       | 13.5| 34         | 125| 12.0       | 125| 12.0       |
| 34.5| 55         | 125| 12.0       | 125| 12.0       |
| 17 | 0          | 0  | 200        |
TABLE V

The effect of Tween 80 detergent in the presence of bovine serum albumin on the microsomal conversion of 7-dehydrocholesterol to cholesterol

| Cholesterol formed | Tween 80 (0.35%) |
|--------------------|------------------|
| Standard assay     | No preincubation | Preincubation |
| nmol/2 h           | 0.4              | 20.0          |

Artificial acceptor/donor, does this finding have physiological meaning? This question was examined using a physiological donor, i.e. adrenal cytoplasmic lipid inclusion droplets. The lipid inclusion droplets were incubated with SCP-2, and the mass of lipids released from the lipid droplets was measured. The results (1) show that cholesterol was the only lipid released from the lipid droplet in the presence of SCP-2. Phospholipid, cholesterol ester, triglyceride, and free fatty acid were not released from the cytoplasmic lipid droplets by SCP-2. These findings (1) strongly support the conclusion that SCP-2 does not play a role in the physiological transfer of lipids other than sterols between organelles.

A recent report (33) on the distribution and diurnal variations of FABP in the adrenal gland is difficult to reconcile in light of the present studies with homogeneous preparations of SCP-2 and FABP. These studies (33) suggest that FABP can attain levels of 10% of the total protein of adrenal homogenates and that 35% of this is associated with mitochondria. Furthermore, data is presented to suggest that mitochondrial FABP is increased under in vivo conditions known to stimulate cholesterol movement to the mitochondrial inner membrane (i.e. ACTH and aminoglutethimide). The present studies clearly demonstrate the specificity of SCP-2 in sequestration of cholesterol from lipid droplets and stimulation of mitochondrial pregnenolone production. We have also shown that SCP-2 can facilitate translocation of cholesterol from mitochondrial outer to inner membranes using mitochondria which are unable to further utilize this cholesterol (blocked with aminoglutethimide) (7).

A possible function for FABP in the adrenal cortex can, however, be anticipated. ACTH can activate adrenal sterol ester hydrolase via the cAMP cascade (34, 35), and this results in hydrolysis of the high levels of endogenous esterified cholesterol of adrenal lipid inclusion droplets (27). Mediation of cholesterol release from these droplets and transfer to mitochondria by SCP-2 has been demonstrated in model systems (6, 8). However, disposition of the fatty acid moiety, resulting from hydrolysis of esterified cholesterol, may be a function of FABP. Delivery of this lipid to mitochondria could account for the high levels of FABP observed in adrenal mitochondria (32). The reason for increased levels of FABP in mitochondria from aminoglutethimide-treated animals, however, is unclear.

In summary, the results of the present investigation show that SCP-2 and FABP have separate and distinct physiological functions, with SCP-2 participating in reactions involving sterols and FABP participating in reactions involving fatty acids. Furthermore, there is no overlap in substrate specificity, i.e. FABP does not possess cholesterol carrier protein activity and SCP-2 does not specifically bind or transport fatty acid.

Thus, to avoid further confusion in nomenclature, we suggest that, in the context of the present studies, the term "sterol carrier protein" be applied only to the M, 13,500 peptide, currently referred to as SCP-2, CM (32), nonspecific lipid transfer protein (36), or nonspecific phospholipid transfer protein (32, 37), and that the term "fatty acid-binding protein" be used to describe the M, 14,200 protein, currently referred to as FABP (11, 38), Z-protein (39, 40), and SCP (9, 16). Furthermore, it is logical to subdivide the term sterol carrier protein into SCP-1 (for the protein required for the microsomal conversion of squalene to lanosterol) and SCP-2 (for the protein required for the conversion of lanosterol to cholesterol, for the conversion of cholesterol to cholesterol ester, and for intracellular cholesterol transfer).

Acknowledgment—We acknowledge the expert technical assistance of Joan A. Manning in the fatty acid-binding studies.

REFERENCES
1. Scallen, T. J., and Vahouny, G. V. (1985) in Comprehensive Biochemistry: Sterols and Bile Acids, in press
2. Srikantaiyah, M. V., Hansbury, R., Loughran, E. D., and Scallen, T. J. (1976) J. Biol. Chem. 251, 5496-5504
3. Gavey, K. L., and Scallen, T. J. (1978) J. Biol. Chem. 253, 5478-5483
4. Ferguson, J. B., and Bloch, K. (1977) J. Biol. Chem. 252, 5381-5385
5. Noland, B. J., Arebalo, R. E., Hansbury, E., and Scallen, T. J. (1980) J. Biol. Chem. 255, 4282-4289
6. Chanderban, R., Noland, B. J., Scallen, T. J., and Vahouny, G. V. (1982) J. Biol. Chem. 257, 8928-8934
7. Vahouny, G. V., Dennis, P., Chanderban, R., Fiskum, G., Noland, B. J., and Scallen, T. J. (1984) Biochem. Biophys. Res. Commun. 122, 509-515
8. Vahouny, G. V., Chanderban, R., Noland, B. J., Irwin, D., Dennis, P., Lambeth, J. D., and Scallen, T. J. (1983) J. Biol. Chem. 258, 11731-11737
9. Dempsey, M. E., McCoy, K. E., Baker, H. N., Dimitriadou-Vafiadou, A. D., Lorsbach, T., and Howard, J. B. (1981) J. Biol. Chem. 256, 1867-1873
10. McGuire, D. M., Olson, D. C., Towie, H. C., and Dempsey, M. E. (1984) J. Biol. Chem. 259, 5368-5371
11. Ockner, R. K., Manning, J. A., and Kane, J. P. (1982) J. Biol. Chem. 257, 7872-7879
12. Vahouny, G. V., Chanderban, R., Noland, B. J., Bass, N. M., Ockner, R. K., and Scallen, T. J. (1984) Fed. Proc. 43, 1900
13. Scallen, T. J., Noland, B. J., Gavey, K. L., Bass, N. M., Ockner, R. K., and Vahouny, G. V. (1984) Fed. Proc. 43, 1901
14. Gabriel, O. (1971) Methods Enzymol. 22, 565-578
15. Blakesley, R. W., and Boezi, J. A. (1977) Anal. Biochem. 82, 580-582
16. Song, M. K. H., and Dempsey, M. E. (1981) Arch. Biochem. Biophys. 211, 523-529
17. Glaz, J. F. C., and Veerkamp, J. H. (1983) Anal. Biochem. 132, 89-95
18. Dahlberg, E., Snochowski, M., and Gustafsson, J. A. (1980) Anal. Biochem. 108, 380-388
19. Gavey, K. L., Noland, B. J., and Scallen, T. J. (1981) J. Biol. Chem. 256, 2993-2999
20. Seubert, W. (1960) Biol. Chem. 277, 580-583
21. Van Vunakis, H., Kaplan, J., Lehrer, R., and Levine, L. (1966) Immunochemistry 3, 393-402
22. Bass, N. M., Manning, J. A., Ockner, R. K., Gordon, J. I., Seetaram, S., and Alpers, D. H. (1965) J. Biol. Chem. 260, 1432-1436
23. Bergon, L., Gallant, S., and Brownie, A. C. (1974) Endocrinology 94, 336-345
24. Keritz, S. B., and Kumar, A. M. (1970) J. Biol. Chem. 245, 152-159
Comparison of SCP-2 and FABP; Separate Physiological Roles

25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265-275
26. Johnson, L. R., Ruhmann-Wennhold, A., and Nelson, D. H. (1973) *Ann. N. Y. Acad. Sci.* 212, 307-318
27. Vahouny, G. V., Chanderbhan, R., Hinds, R., Hodges, V. A., and Treadwell, C. R. (1978) *J. Lipid Res.* 19, 570-577
28. Burnett, D. A., Lysenko, N., Manning, J., and Ockner, R. K. (1979) *Gastroenterology* 77, 241-249
29. Daum, H. A. (1979) PhD thesis, University of Minnesota
30. Mishkin, S., Stein, L., Gatmaitan, Z., and Arias, I. M. (1972) *Biochern. Biophys. Res. Commun.* 47, 997-1003
31. Ishibashi, T., and Bloch, K. (1981) *J. Biol. Chem.* 256, 12962-12967
32. Bloj, B., Hughes, M. E., Wilson, D. B., and Zilversmit, D. B. (1978) *FEBS Lett.* 96, 87-89
33. Conneely, O. M., Headon, D. R., Olson, C. D., Ungar, F., and Dempsey, M. E. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 2970-2974
34. Beckett, G. J., and Boyd, G. S. (1977) *Eur. J. Biochem.* 72, 223-233
35. Nashshineh, S., Treadwell, C. R., Gallo, L. L., and Vahouny, G. V. (1978) *J. Lipid Res.* 19, 561-569
36. Trzaskos, J. M., and Gaylor, J. L. (1983) *Biochim. Biophys. Acta* 751, 52-65
37. Poorthius, B. J. H. M., Glasta, J. F. C., Akeroyd, R., and Wirtz, K. W. A. (1981) *Biochim. Biophys. Acta* 665, 256-261
38. Ockner, R. K., and Manning, J. A. (1974) *J. Clin. Invest.* 54, 326-338
39. Levi, A. J., Gatmaitan, Z., and Arias, I. M. (1969) *J. Clin. Invest.* 48, 2156-2167
40. Mishkin, S., and Turcotte, R. (1974) *Biochem. Biophys. Res. Commun.* 57, 918-926.