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Nasal absorption of insulin: Enhancement by hydrophobic bile salts

(drug absorption/hydrophobicity/mixed micelles/reverse micelles/steroid detergents)

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ABSTRACT **We demonstrate that therapeutically useful amounts of insulin are absorbed by the nasal mucosa of human beings when administered as a nasal spray with the common bile salts. By employing a series of bile salts with subtle differences in the number, position, and orientation of their nuclear hydroxyl functions and alterations in side chain conjugation, we show that adjuvant potency for nasal insulin absorption correlates positively with increasing hydrophobicity of the bile salts' steroid nucleus. As inferred from studies employing various concentrations of unconjugated deoxycholate and a constant dose of insulin, insulin absorption begins at the aqueous critical micellar concentration of the bile salt and becomes maximal when micelle formation is well established. These and other data are consistent with the complementary hypotheses that bile salts act as absorption adjuvants by (*i*) producing high juxtamembrane concentrations of insulin monomers via solubilization in mixed bile salt micelles and (*ii*) forming reverse micelles within nasal membranes, through which insulin monomers can diffuse through polar channels from the nares into the blood stream.**

Certain small peptides can be absorbed through the nasal mucosa as a "snuff" or directly from aqueous solution (1–5). However, efficacy of absorption is typically low and variable (1–5), and therapeutically important peptides of larger molecular size, such as insulin, are not absorbed to any appreciable degree (6). Within the gastrointestinal tract, bile salts promote the transmembrane movement of endogenous and exogenous lipids (7) and the transmembrane and/or paracellular movement of several small endogenous and exogenous polar molecules—e.g., water (7), inorganic electrolytes (7), polyethylene glycols (8), and oxalate (9). Because of these functions, as well as their detergent-like properties on biomembranes (10), bile salts are potential adjuvants for transmucosal delivery of drugs and have been widely explored for this purpose (11–17). Although there is abundant physical-chemical information concerning the micellar properties of bile salt molecules as well as their interactions with membrane and exogenous lipids (18, 19), little is known about the mechanisms by which these molecules might enhance transmucosal absorption of drugs (17). As shown by us and others, bile salts promote the nasal absorption of insulin in man (20, 21) as well as in laboratory animals (6, 22). Nevertheless, previous studies in rats, employing a range of bile salt species, failed to define any useful structure–function relationships (23). We now report structure–function studies on a series of naturally occurring bile salts by testing their ability to enhance insulin absorption across the human nasal mucosa when administered intranasally as an insulin/bile salt spray. Dramatic differences in insulin absorption were observed between closely related bile salt species; the pattern was shown to be determined by the hydrophilic–hydrophobic balance (24) of the hydroxyl-substituted steroid nucleus and not that of the overall molecule. This correlation suggests strategies for future development of safe and effective insulin-transporting agents.

MATERIALS AND METHODS

Experimental Subjects. We studied 40 healthy human volunteers 19–35 years old who were within 10% of ideal body weight. All subjects gave written informed consent to an experimental protocol approved by the Clinical Investigation Committee of Beth Israel Hospital and were studied in the hospital's Clinical Research Center. Subjects were studied in the supine position on the morning after an overnight fast. Intravenous catheters were placed in a forearm vein for blood drawing; patency was ensured by the continuous infusion of 0.15 M NaCl at the rate of 15 ml/hr.

Insulin and Bile Salts. Commercially available U-500 regular porcine insulin was obtained from Eli Lilly. One unit (U) of insulin = 42 μg. Bile salts [sodium salts of deoxycholate, glycodeoxycholate, taurodeoxycholate (all 3α,12α-dihydroxy-5β-cholanoates); sodium salts of cholate, glycocholate, and taurocholate (all 3α,7α,12α-trihydroxy-5β-cholanoates)] were purchased from Calbiochem and were purified by recrystallization according to the methods of Pope (25) and Norman (26). Bile acids [ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholanoic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid)] were received as generous gifts from Herbert Falk (Falk GmbH, Freiburg, Federal Republic of Germany) and were converted to the sodium salts as described (24). Purity of all bile salts was greater than 98–99% as determined by thin-layer chromatography, reverse-phase high-performance liquid chromatography (HPLC), and titration with HCl (24, 27).

Methods. Immediately prior to use, bile salts were dissolved in 0.15 M NaCl at pH 10 to give concentrations of 2–5% (wt/vol). With 1 M HCl, the pH of each solution was adjusted to 7.4–7.8 except for that of ursodeoxycholate, which was adjusted to 8.1 owing to its insolubility at physiologic pH (27). Bile salt solutions were mixed with U-500 regular porcine insulin in 0.15 M NaCl to give final bile salt concentrations of 1% (wt/vol) and sufficient insulin for an intended delivery of 0.5 U/kg of body weight. Since the volume of the administered spray was fixed, and the weight of subjects varied from 40 to 85 kg, the ratio of bile salt to insulin varied over a 2-fold range. Insulin/bile salt solutions were administered within 2 hr of mixing as single sprays in each nostril, employing a metered-pump sprayer (Boehringer Ingelheim, Ridgefield, CT), which delivered 75 ± 8 μl per spray.

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Abbreviations: U, unit; cmc, critical micellar concentration.

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Venous blood was drawn at appropriate time intervals and allowed to clot at room temperature before centrifugation at 2000 rpm for 30 min in an International model PR-2 at 4°C. Serum was stored at −20°C for less than 1 week. After thawing at 23°C, samples from individual subjects were assayed for insulin in a single batch to avoid interassay variations. Serum insulin levels were measured by coated tube radioimmunoassay kits (Clinical Assays, Division of Baxter-Travenol, Cambridge, MA).

THEORETICAL CONSIDERATIONS

Armstrong and Carey (24) demonstrated that the cholesterol-solubilizing capacity of bile salt micelles correlates inversely with the hydrophilic–hydrophobic balance of the bile salt monomer: the most hydrophobic bile salts had the greatest capacities to solubilize cholesterol. It also was demonstrated that the hydrophilic–hydrophobic balance of a bile salt can be predicted on the basis of the bile salt's retention factor (k') in reverse-phase HPLC employing a μBondapak C_{18} column (Altex, Palo Alto, CA) (24). In this report, we utilized the same HPLC configuration and eluted the bile salts with a mobile phase composed of 75% methanol/25% water with 0.005 M phosphate buffer, pH 5.4 (vol/vol). We calculated k' for each bile salt according to the formula

$$k' = \frac{t_r - t_o}{t_o},$$

in which t_o = retention time of the solvent front and t_r = retention time of the bile salt (24). We correlated the hydrophilic–hydrophobic balance of the bile salts studied with their capacity to deliver into the systemic circulation a fixed dose (0.5 U/kg, range 20–43 U, depending upon body weight) of regular porcine insulin sprayed intranasally.

RESULTS

Fig. 1 demonstrates that nasal absorption of insulin correlates positively with hydrophobicity of the unconjugated bile salts as inferred from their HPLC retention factors (k') (24), with the rank ordering being deoxycholate > chenodeoxycholate > cholate > ursodeoxycholate. With bile salt concentrations of 1 g/dl, all serum insulin levels peaked at 10 ± 1 min and blood glucose fell in parallel with these changes (not displayed). As determined by the area under the serum insulin vs. time curves, 10–20% of the administered dose of insulin was absorbed into the circulation in the presence of 1% wt/vol (24 mM) sodium deoxycholate (20). Whereas the most hydrophilic bile salt, sodium ursodeoxycholate, did not appreciably promote insulin absorption (Fig. 1) or lower blood glucose concentration (20), the most hydrophobic bile salt, sodium deoxycholate, produced marked elevations in serum insulin concentrations (Fig. 1) and ≈50% decreases in blood glucose concentrations (20).

When bile salts are conjugated with glycine or taurine, the overall hydrophobicity of the molecules decreases because of the highly charged amino acid side chains, yet adjuvant activity is retained (Fig. 1). This suggests that the hydrophobicity of the steroid nucleus, and not that of the overall molecule, is the major determinant of adjuvant activity. The rank ordering of bile salt potency as well as the lack of an appreciable difference between unconjugated bile salts and their conjugates are paralleled by their effects on other biomembranes such as stimulation of water and electrolyte secretion by the colon (28).

The unconjugated bile salts studied in this work self-associate in dilute aqueous (0.15 M Na^+) solutions to form small polymolecular aggregates, called micelles, at a critical micellar concentration (cmc) of approximately 3–7 mM at

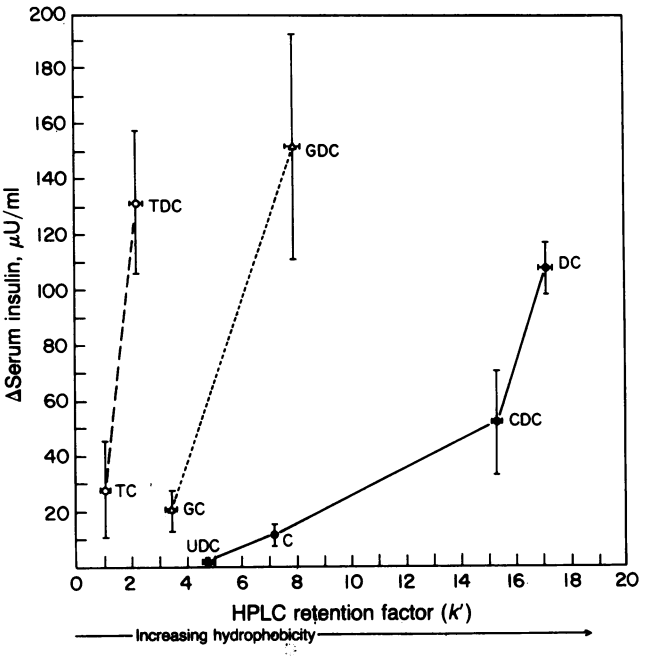

FIG. 1. Dependence of peak serum insulin on bile salt hydrophobicity. HPLC retention factor (k') was calculated according to Armstrong and Carey (24) and increases with increasing hydrophobicity. Insulin was administered at a dose of 0.5 U/kg of body weight. Deoxycholate (DC), chenodeoxycholate (CDC), cholate (C), ursodeoxycholate (UDC), glycodeoxycholate (GDC), glycocholate (GC), taurodeoxycholate (TDC), and taurocholate (TC) were administered at final concentrations of 1% (wt/vol). Results are given as mean ± SEM.

23–25°C (18, 29–33). However, bile salts are different from flexible-chain detergents in that initial self-association is only a mildly cooperative process, and aggregation continues with increasing concentration (33, 34). In these micelles, the hydrophobic sides of the molecules are oriented inwards, whereas the hydrophilic sides face outwards toward the aqueous environment (18, 19, 35). To explore whether the aqueous self-association of bile salts is an important determinant of adjuvant activity, we investigated the relationship between concentration of sodium deoxycholate (0–24 mM at pH 7.8–8.2) and the resultant peak serum insulin concentration after identical insulin doses on a kg basis (Fig. 2). The sigmoid dose–response curve shows that sodium deoxycholate was only slightly effective at 2.5 mM, 50% effective at 6 mM, and maximally effective at 12 and 24 mM. Since the aqueous cmc of deoxycholate in 0.15 M NaCl is approximately 3 mM (18, 31), these data imply that, to exhibit optimal adjuvant activity, deoxycholate must be well above its *aqueous* cmc. As demonstrated in the Fig. 2 *Inset*, increasing deoxycholate concentrations from 6 to 12 mM influenced the magnitude of the serum insulin peak but not the time at which the peak occurred. Since the cmc values of sodium chenodeoxycholate, sodium ursodeoxycholate, and sodium cholate are approximately 3, 5, and 7 mM, respectively, under the same experimental conditions (18, 29–33), the reduced or absent activity of these bile salts when compared with deoxycholate is not wholly a function of their aqueous cmc values.

DISCUSSION

In this report, we have attempted to relate a fundamental physicochemical characteristic of bile salt molecules, that is their hydrophilic–hydrophobic balance, to their capacity to enhance insulin absorption through the nose. The hydrophilic–hydrophobic balance of these detergent-like molecules in

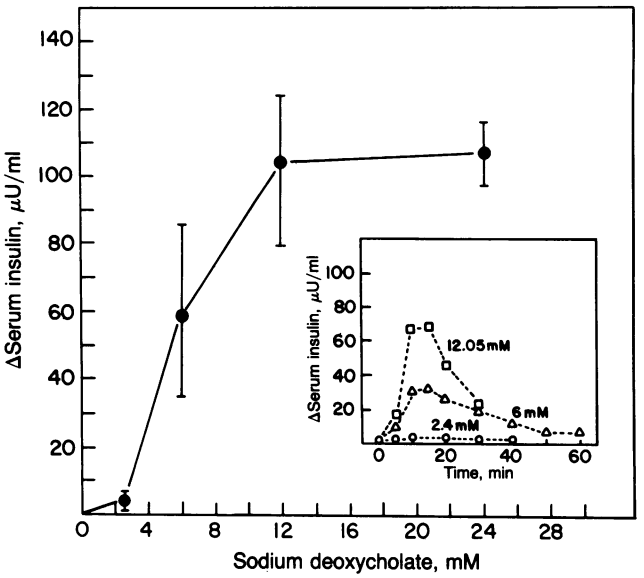

FIG. 2. Dependence of peak serum insulin on concentration of sodium deoxycholate. Peak increment in serum insulin is plotted versus the final concentration of sodium deoxycholate in the nasal spray. The equivalent of 1% (wt/vol) sodium deoxycholate is 24 mM. Insulin was administered at a dose of 0.5 U/kg. (*Inset*) Kinetics of insulin absorption at three different concentrations of sodium deoxycholate. Results are presented as mean ± SEM.

turn influences their aqueous cmc values and membrane–water partitioning coefficients (33). In this work, we have observed a striking correlation not only between bile salt hydrophobicity (24) and adjuvant activity (Fig. 1) but also between aqueous micelle formation [which continues to change over a wide range of concentrations (34)] and adjuvant activity (Fig. 2).

Our observations are distinctly different from those of Hirai *et al.* (22), who studied the effects of several bile salts and other ionic or nonionic detergents on insulin absorption across the nasal mucosa of rats. These investigators found equivalent activity with sodium cholate, sodium chenodeoxycholate, and sodium deoxycholate, whereas sodium ursodeoxycholate was not studied. The different results obtained in the present work compared to those of Hirai *et al.* (22) may be related to the use of commercial preparations of bile salts, which often are contaminated with other closely related, usually more hydrophobic, species, and to differences in experimental design. These authors allowed insulin/bile salt solutions to have prolonged contact with the nasal mucosa of rats after occlusion of the anterior and posterior nares; hence, only cumulative decreases in blood glucose were observed. Thus, if maximal insulin transport varied with different bile salt species, the experimental design employed by these authors would have obscured this effect.

In a more recent study, Murakami *et al.* (17) found a poor correlation between bile salt-enhanced absorption of sodium ampicillin from rectal pouches of rats and thin-layer chromatographic indices of bile salt hydrophobicity which were, in part, theoretically derived. Further, the rank ordering of the potency of the unconjugated bile salt species was chenodeoxycholate > deoxycholate >> ursodeoxycholate > cholate. Nonetheless, the dose–response relationship of Murakami *et al.* (17) for peak blood ampicillin level as a function of chenodeoxycholate concentration was in striking agreement with our work (Fig. 2); enhancement of absorption began at the chenodeoxycholate concentration corresponding to its aqueous cmc (≈3 mM) and reached maximal values at 12 mM.

The mechanism by which bile salts enhance absorption of insulin (or other drugs) across biomembranes is not known. To be absorbed from the nasal mucosa to reach the blood circulation, insulin molecules must be transported through (or between) a number of membrane barriers in series. These include apical and basal membranes of mucosal cells, the lamina propria, and capillary endothelial cells (36). At physiologic pH, insulin molecules are only sparingly soluble as monomers in aqueous systems (37) and in biomembranes (38) (≈170 pM). Interestingly, such insulin concentrations approximate both basal and stimulated physiological blood levels (39, 40). Solutions of so-called "soluble" insulin in various commercial formulations contain particles that range in size from ≤ 100 Å to >100,000 Å (41), suggesting that a high proportion of aqueous insulin molecules are present as either microcrystals or polymers. This self-aggregation process occurs because the exterior of the insulin monomer, as shown crystallographically, has two large nonpolar (hydrophobic) surfaces on opposing sides (42); one side is involved in dimer formation, and the other in higher aggregate (hexamer–polymer) formation (42). As determined by quasielastic light scattering, 1% (wt/vol) micellar concentrations of each unconjugated bile salt studied herein completely solubilized 1% (wt/vol) insulin by forming mixed micelles of bile salt and insulin molecules. When compared with the identical bile salt solutions without insulin, the degree of micellar expansion induced by insulin corresponded to micellar solubilized insulin monomers (unpublished observations). Mixed micelle formation most likely provides a high juxtamembrane concentration (in our formulations, ≈1–2 mM) of soluble insulin that facilitates the flow of insulin monomers down a concentration gradient from the nares into the nasal membranes. These considerations are the most likely explanation for the dose–response curve in Fig. 2, where bile salt adjuvant activities became unmasked only above the aqueous cmc of the deoxycholate and leveled off at concentrations customarily accepted as corresponding to well-developed stepwise self-association (34).

Because 1% wt/vol of each bile salt completely solubilized 1% wt/vol insulin as monomers, we believe, on the basis of other studies (unpublished observations), that the differing adjuvant activities of various bile salt species relates to their differing capacities to penetrate and self-associate as reverse micelles within native membranes (hydrophobic bile salts >> hydrophilic bile salts) as they do in nonpolar solvents or when dispersed in pure phospholipid environments (10, 19, 33). In reverse micelles, the hydrophilic surfaces of the molecules face inwards and the hydrophobic surfaces face outward toward the lipid environment (Fig. 3). Thus, reverse micelles could act as transmembrane channels or mobile carriers for insulin to move down an aqueous concentration gradient through the nasal mucosal cells, into the intercellular space, and into the blood stream (Fig. 3). While this mode of intramembranous bile salt self-association has been invoked to explain the high solubility of bile salts in membranes and synthetic bilayers (10, 19), its functional significance has only recently been addressed. Hunt (43) and Castallino and Violand (44), employing NMR spectroscopy, have suggested that reverse micelles of bile salt molecules account for the rapid transport of lanthanides into unilamellar vesicles, and Hunt (43) suggested, on the basis of kinetic analysis, that transmembrane diffusion of lanthanide/bile salt complexes occurred. However, our study and that of Murakami *et al.* (17) show transport saturation at bile salt concentrations of ≈12 mM (Fig. 2). This finding argues for local membrane saturation with the steroid detergent and a channel-type reverse micelle mechanism rather than a mobile reverse micelle carrier. The far greater adjuvant potency of the more hydrophobic dihydroxy bile salts with two α-oriented hydroxyl functions compared with cholate and ursodeoxy-

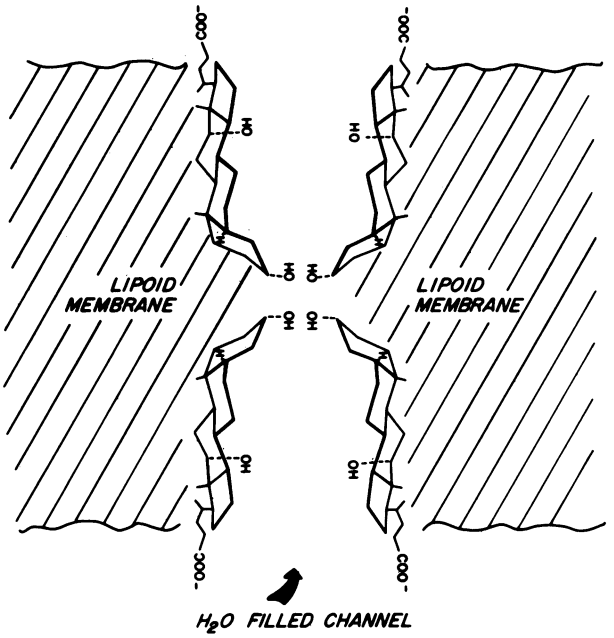

FIG. 3. A schematic molecular model of reverse micelle formation in cell membranes. Two pairs of sodium deoxycholate molecules are shown stacked end-to-end, spanning a lipid bilayer and forming an aqueous pore for the transport of insulin monomers from the extracellular space, where high concentrations of insulin monomers are solubilized in mixed bile salt/insulin micelles (see text for discussion).

cholate could be related, in part, to larger sizes of the reverse micelles formed by the former species, which would facilitate the formation of more capacious water-filled intramembranous pores. Further, intramolecular hydrogen bonding in reverse micelles of cholate is extremely tight, thus the free water core may be restricted. In the case of ursodeoxycholate, the β-oriented hydroxyl at C7 on the steroid skeleton impairs reverse micelle formation (reviewed in ref. 33).

The molecular dimensions of lipid bilayers and the steroid nucleus and extended polar side chain of the bile salts (18, 19) suggest that pairs of bile salt reverse micelles stacked end-to-end could span biomembrane bilayers, with the ionized polar groups projecting into the aqueous environment on either side (Fig. 3). Preliminary studies of the high degree of partitioning of biologically active insulin molecules into reverse micelles of deoxycholate in water/decanol systems and the efficient aqueous-to-aqueous cotransport of monomeric insulin with bile salts through a bulk chloroform phase in a forced convection apparatus provide strong support for this explanation (unpublished observations).

Other possible mechanisms by which bile salts could promote transmembrane transport of insulin include the binding of Ca^{2+}, which would loosen tight junctions between cells (45). Even though Ca^{2+} binding increases with bile salt hydrophobicity, it is reduced by bile salt conjugates compared with the unconjugated species (46) and would be unlikely to explain the results in Fig. 1. Steroid detergents also decrease the "order" of lamellar liquid-crystalline phases (47) and, by implication, membranes, and may therefore allow bulk water/polypeptide movement through a loosely ordered membrane structure. Alternatively, high local bile salt concentrations, by inducing regional phase changes within membranes to form cubic and hexagonal liquid-crystalline structures (19, 48), could provide a network of water-filled channels (47) through which water-soluble molecules could diffuse. For example, Ericsson *et al.* (49) have shown that proteins ranging in molecular weight from 5000 to 150,000 can be incorporated into and diffuse through water channels of a cubic phase formed by monoolein/water systems. Finally, Hirai *et al.* (23) have suggested that bile salts may promote insulin transport across the nasal mucosa by retarding insulin degradation by leucine aminopeptidase, a proteolytic enzyme of the nasal mucosa. When a 20-fold excess of tyrosyltyrosine, an alternative substrate for this enzyme was added, the dipeptide did not influence the extent of insulin absorption from the bile salt nasal spray (unpublished observations). Obviously, more work on the mechanisms involved in transmembrane transport of insulin by bile salts is needed.

A prime objective in developing an adjuvant for the transmucosal administration of insulin and other polypeptides is to identify an effective membrane-homing surfactant that does not cause local or systemic toxicity. Each of the unconjugated bile salts tested in this study produced local nasal irritation as assessed by a brief (3- to 5-min) burning sensation in the nose. However, this irritation did not correlate with the adjuvant activity of the bile salt, since ursodeoxycholate, which was inactive, was the most irritating. Taurine and glycine conjugates of the bile salts were somewhat less irritating to the nasal mucosa, as they are in terms of toxicity on other membranes (7). The observation that adjuvant activity of bile salts for transmembrane insulin transport can be predicted on the basis of the hydrophilic–hydrophobic balance of the monomers, a property easily measured (24), raises the possibility that bile salts or related molecules can be structurally engineered to retain adjuvant activity without producing potentially toxic side effects.

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1. Dashe, A. M., Kleeman, C. R., Czarczkes, J. W., Rubinoff, H. & Spears, I. (1964) *J. Am. Med. Assoc.* **190**, 113–115.
2. Baumann, G., Walser, A., Desaulles, P. A., Paesi, F. J. A. & Geller, L. (1975) *J. Clin. Endocrinol. Metab.* **42**, 60–63.
3. Solbach, H. G. & Wiegelmann, W. (1973) *Lancet* **i**, 1259.
4. Evans, W. S., Borges, J. L. C., Kaiser, D. L., Vance, M. L., Sellers, R. P., MacLeod, R. M., Vale, W., Rivier, J. & Thorner, M. O. (1983) *J. Clin. Endocrinol. Metab.* **57**, 1081–1083.
5. Altszuler, N. & Hampshire, J. (1981) *Proc. Soc. Exp. Biol. Med.* **168**, 123–124.
6. Hirai, S., Ikenaga, T. & Matsuzawa, T. (1978) *Diabetes* **27**, 296–299.
7. Carey, M. C. (1982) in *The Liver: Biology and Pathobiology*, eds. Arias, I. M., Popper, H., Schacter, D. & Shafritz, D. (Raven, New York), pp. 429–465.
8. Tagesson, C. & Sjödahl, R. (1984) *Eur. Surg. Res.* **16**, 274–281.
9. Dobbins, J. W. & Binder, H. J. (1976) *Gastroenterology* **70**, 1096–1100.
10. Helenius, A. & Simons, K. (1975) *Biochim. Biophys. Acta* **415**, 29–79.
11. Ziv, E., Kidron, M., Berry, E. M. & Bar-on, H. (1981) *Life Sci.* **29**, 803–809.
12. Ziv, E., Eldor, A., Kleinman, Y., Bar-on, H. & Kidron, M. (1983) *Biochem. Pharmacol.* **32**, 773–776.
13. Gibaldi, M. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1343–1349.
14. Hollander, D., Dadufalza, V. D. & Fairchild, P. A. (1981) *J. Lab. Clin. Med.* **98**, 591–598.
15. Garcin-Salomon, C., Boucherat, J., Crevoisier, C. & Buri, P. (1980) *Pharm. Acta Helv.* **55**, 78–81.
16. Kocsár, L. T., Bertók, L. & Várterész, V. (1969) *J. Bacteriol.* **100**, 220–223.

17. Murakami, T., Sasaki, Y., Yamajo, R. & Yata, N. (1984) *Chem. Pharm. Bull.* **32,** 1948–1955.
18. Carey, M. C. (1983) in *Bile Acids in Gastroenterology*, eds. Barbara, L., Dowling, R. H., Hofmann, A. F. & Roda, E. (MTP, Boston), pp. 19–56.
19. Small, D. M. (1971) in *The Bile Acids*, eds. Nair, P. P. & Kritchevsky, D. (Plenum, New York), Vol. 1, pp. 249–356.
20. Moses, A. C., Gordon, G. S., Carey, M. C. & Flier, J. S. (1983) *Diabetes* **32,** 1040–1047.
21. Pontiroli, A. E., Alberetto, M., Secchi, A., Dossi, G., Bisi, I. & Pozza, G. (1982) *Br. Med. J.* **284,** 303–306.
22. Hirai, S., Yashiki, T. & Mima, H. (1981) *Int. J. Pharm.* **9,** 165–172.
23. Hirai, S., Yashiki, T. & Mima, H. (1981) *Int. J. Pharm.* **9,** 173–184.
24. Armstrong, M. J. & Carey, M. C. (1982) *J. Lipid Res.* **23,** 70–80.
25. Pope, J. L. (1967) *J. Lipid Res.* **8,** 146–147.
26. Norman, A. (1955) *Ark. Kemi* **8,** 331–342.
27. Igimi, H. & Carey, M. C. (1980) *J. Lipid Res.* **21,** 72–90.
28. Mekhjian, H. S., Phillips, S. F. & Hofmann, A. F. (1971) *J. Clin. Invest.* **50,** 1569–1577.
29. Carey, M. C., Montet, J.-C. & Small, D. M. (1975) *Biochemistry* **14,** 4896–4905.
30. Carey, M. C., Montet, J.-C., Phillips, M. C., Armstrong, M. J. & Mazer, N. A. (1981) *Biochemistry* **20,** 3637–3648.
31. Roda, A., Hofmann, A. F. & Mysels, K. J. (1983) *J. Biol. Chem.* **258,** 6362–6370.
32. Hisadome, T., Nakama, T., Itoh, H. & Furusawa, T. (1980) *Gastroenterol. Jpn.* **15,** 257–263.
33. Carey, M. C. (1985) in *New Comprehensive Biochemistry*, eds. Sjövall, J. & Danielsson, H. (Elsevier, Amsterdam), Vol. 12, pp. 345–403.
34. Mukerjee, P., Moroi, Y., Murata, M. & Yang, A. Y. S. (1984) *Hepatology (NY)* 4:61S–65S.
35. Mazer, N. A., Carey, M. C., Kwasnick, R. F. & Benedek, G. B. (1979) *Biochemistry* **18,** 3064–3075.
36. Cauna, N. (1982) in *The Nose: Upper Airway Physiology and the Atmospheric Environment*, eds. Proctor, D. F. & Anderson, I. (Elsevier, Amsterdam), pp. 45–69.
37. Windholz, M., ed. (1983) *The Merck Index* (Merck, Rahway, NJ), 12th Ed., pp. 723–724.
38. Hyslop, P. A., York, D. A., Sauerheber, R. D. (1984) *Biochim. Biophys. Acta* **776,** 267–278.
39. Berson, S. A., Yalow, R. S. (1966) *Am. J. Med.* **40,** 676–690.
40. Cahill, G. F., Jr. (1971) *Diabetes* **20,** 785–799.
41. Martindale, H., Marsh, J., Hallett, F. R. & Albisser, A. M. (1982) *Diabetes* **31,** 364–366.
42. Blundell, T. L., Cutfield, J. F., Cutfield, S. M., Dodson, E. J., Dodson, G. G., Hodgkin, D. C. & Mercola, D. A. (1972) *Diabetes* **21,** 492–505.
43. Hunt, G. R. A. (1980) *FEBS Lett.* **119,** 132–136.
44. Castallino, F. J. & Violand, B. N. (1979) *Arch. Biochem. Biophys.* **193,** 545–550.
45. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D., eds. (1983) *Molecular Biology of the Cell* (Garland, New York), pp. 673–716.
46. Kahn, M. J., Lakshminarayanaiah, N., Trotman, B. W., Chun, P., Kaplan, S. A. & Margulies, C. (1982) *Hepatology (NY)* **2,** 732 (abstr.).
47. Ulmius, J., Lindblom, G., Wennerström, H., Johansson, L. B.-Å., Fontell, K., Söderman, O. & Arvidson, G. (1982) *Biochemistry* **21,** 1553–1560.
48. Small, D. M., Bourgès, M. & Dervichian, D. G. (1966) *Nature (London)* **211,** 816–818.
49. Ericsson, B., Larsson, K. & Fontell, K. (1983) *Biochim. Biophys. Acta* **729,** 23–27.