Discrimination between cholesterol and sitosterol for absorption in rats

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Abstract The intestinal absorption of cholesterol and sitosterol was compared in rats. The intragastric administration of a single emulsified lipid meal containing either 50 mg of [4,14C]cholesterol or [4,14C]sitosterol resulted in the lymphatic absorption of 18.2% and 0.42% of each sterol, respectively, in 6 hr. This difference was unaltered when the mucosal sterol load was equalized by reducing the cholesterol to 1 mg in the emulsified lipid meal while maintaining the same sitosterol load or when the physical state in the lumen was equalized by infusion of a micellar solution containing both sterols into bile-diverted intestine. Lymphatic cholesterol was 90% esterified compared to 12% for sitosterol. Both sterols were associated predominantly (>70%) with the chylomicron fraction. Eighty percent of the chylomicron cholesterol was recovered as ester with the chylomicron coat. In mucosal homogenates at 6 hr, sitosterol recovery was one-eleventh that of cholesterol. When [3H]cholesterol (10 mg) and [14C]sitosterol (10 mg) were co-administered in an emulsified intragastric lipid meal, sitosterol associated with the brush border isolated 2 hr later was one-fifth that of cholesterol. Similar differences were seen when brush border membranes were incubated in vitro with micellar solutions containing either 50 μM [3H]cholesterol or [14C]sitosterol and the relative uptake of each sterol was unaffected by micellar phospholipid type (egg yolk phospholipids, phosphatidylcholine, or phosphatidylethanolamine). When the affinity of several absorbable and nonabsorbable sterols for the mixed taurocholate micelle was compared in an in vitro model system which assessed sterol movement from the micellar to the oil phase, there was preferential movement of cholesterol and other absorbable sterols whereas nonabsorbable sterols including sitosterol were retained. The differential sterol affinity was dependent on the bile salt composition of the micelle with the glycine or taurine conjugates of cholate most effective. Transfer of the two sterols was equivalent from Triton X-100 and phospholipid emulsions. These studies suggest that the mechanism that accounts for inhibition of cholesterol absorption is distinct from that which discriminates between cholesterol and plant sterols for absorption. Specifically, sitosterol (6, 7) and fucosterol (7) displaced cholesterol from micellar solution accounting for the inhibition of its absorption, but micellar solubilization does not insure absorption since sitosterol is not absorbed even where fully micellar-solubilized (7). Several potential sites for discrimination have been suggested. One is at the level of mucosal sterol esterification and is based on the repeated observations that, in contrast to cholesterol, sitosterol appears largely unesterified in the lymph of experimental animals (1–3). This observation is supported by in vitro studies that demonstrate that sitosterol is esterified less well (3, 8–10) than cholesterol by the intestinal esterification enzymes, cholesterol esterase (7) and ACAT (11). Although sitosterol is a poor substrate for esterification, this may be relatively unimportant in discrimination if sitosterol is not

Supplementary key words plant sterols • hypocholesterolemia • brush border membranes • sterol esterification • bile salts • phospholipids • mixed micelles • lymph cannula

In contrast to cholesterol, plant sterols are poorly absorbed (1–3). From a structural standpoint, nonabsorbability is most clearly related to substitutions in position 24 (4, 5) on the sterol side chain with methyl or ethyl groups or their unsaturated counterparts as exemplified by campesterol (24-methyl), sitosterol (24-ethyl), stigmasterol (Δ22, 24-ethyl), and fucosterol (24-ethylidene). Each of these is poorly absorbed and inhibits cholesterol absorption. Present data suggest that the mechanism that accounts for inhibition of cholesterol absorption is distinct from that which discriminates between cholesterol and plant sterols for absorption. Specifically, sitosterol (6, 7) and fucosterol (7) displaced cholesterol from micellar solution accounting for the inhibition of its absorption, but micellar solubilization does not insure absorption since sitosterol is not absorbed even where fully micellar-solubilized (7). Several potential sites for discrimination have been suggested. One is at the level of mucosal sterol esterification and is based on the repeated observations that, in contrast to cholesterol, sitosterol appears largely unesterified in the lymph of experimental animals (1–3). This observation is supported by in vitro studies that demonstrate that sitosterol is esterified less well (3, 8–10) than cholesterol by the intestinal esterification enzymes, cholesterol esterase (7) and ACAT (11). Although sitosterol is a poor substrate for esterification, this may be relatively unimportant in discrimination if sitosterol is not

Abbreviations: C, cholesterol; S, sitosterol; BSA, bovine serum albumin; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); ACAT, acyl coenzyme A:cholesterol acyltransferase; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; MO, monooenol; OA, oleic acid; BS, bile salt; G(1)C, G(1)TDC, G(1)TDC, glycine (G) taurine (T) conjugates of cholic, chenodeoxycholic, and deoxycholic acids; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

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delivered to the esterification enzymes. Related to this, reports on the extent of sitosterol uptake by the intestine in vivo are not in agreement. For example, Swell et al. (12) reported that sitosterol was taken up by the intestinal wall. In contrast, Borgström (13) did not observe an accumulation of administered sitosterol in the intestinal wall and suggested that the distinction between absorbable and nonabsorbable sterols occurred during the process of uptake into the mucosa. Our recent results (7) support the latter finding.

Several studies support discrimination between cholesterol and sitosterol at the brush border membrane. Child and Kuksis (14), using the 7-dehydro analogs of the two sterols in micellar solution, reported a 4- to 5-fold uptake differential by rat brush border membranes in vitro, and Ikeda and Sugano (15) reported the intestinal uptake of sitosterol intubated into the stomach of rats was about one-fifth that of cholesterol. The available combined data leave open the possibility that cellular uptake at the level of the brush border may represent a major site of discrimination and the effect by failing to esterify even the limited amount of possibility that cellular uptake at the level of the brush border may represent a major site of discrimination and intracellular steps beyond, e.g., esterification, may amplify the effect by failing to esterify even the limited amount of sitosterol available.

Not yet tested is the possibility that absorbable and unabsorbable sterols may express different affinities for the micelle as suggested in the recent studies of Armstrong and Carey (16), and thus their rate of delivery to the cell surface may differ.

The present studies were conducted to reexamine the unsettled question of the mechanism of discrimination between cholesterol and sitosterol for lymphatic absorption. Their luminal and intracellular behavior are compared in a combination of in vivo and in vitro studies.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA) fraction V (fatty acid-poor), triolein (>99% purity, no sterol), monoolein, oleic acid (>99% purity), fucosterol (>95% purity), egg yolk phospholipids, phosphatidylethanolamine, phosphatidylcholine, and dioleoyl and dipalmitoyl phosphatidylcholine, and 7-dehydrocholesterol were obtained from Sigma Chemical Co., St. Louis, MO. Cholesterol (>99% purity) was purchased from Serdary Research Laboratories, Ontario, Canada. Sitosterol, from ICN Pharmaceuticals, Cleveland, OH, was recrystallized from ethylacetate-methanol to >98% purity. Stigmasterol, campesterol, and cholestanol were gifts from Dr. William Connor and displayed purity. Sterols occurred during the process of uptake into the mucosa. Their lumenal and intracellular behavior are compared in a combination of in vivo and in vitro studies.

Animal procedures

The presurgical, surgical, and postsurgical treatments of the rats are precisely as described previously (7).

Preparation of lipid meals and “artificial” bile for in vivo sterol absorption studies

A lipid emulsion for intragastric administration in the sterol absorption studies was prepared as described earlier (7) and contained per 3 ml of physiologic saline: 50 mg BSA, 292 mg oleic acid, 279 mg sodium taurocholate, and 50 mg of either [4-14C]cholesterol or [4-14C]sitosterol or 1 mg of [4-14C]cholesterol. For the studies of sterol incorporation into brush border membranes in vivo the emulsion was modified to contain 20 mg of [3H]cholesterol plus 20 mg of [4-14C]sitosterol in the 3.0 ml volume. These were prepared immediately before use and rehomogenized before administration.

Sterol-containing micellar solutions were prepared as previously described (7) for intraduodenal infusion in the sterol absorption studies and contained 6.6 mM sodium taurocholate, 0.6 mM egg yolk phospholipids, and 20-200 µM [4-14C]cholesterol and/or [4-14C]sitosterol in 15 mM sodium phosphate buffer, pH 7.4, containing 62 mM NaCl and 2.5% glucose.

A sterol-free “artificial” bile was prepared as described previously (7).

Preparation of micellar solutions for in vitro studies

A series of micellar solutions were prepared for the in vitro studies. These varied in the number and type of component, as well as in the concentration of sterol. Each contained 6.6 mM bile salt (the glycine or taurine conjugates of cholate, deoxycholate, or chenodeoxycholate) and 20-200 µM [4-14C]cholesterol, sitosterol, fucosterol, campesterol, stigmasterol, cholestanol, or 7-dehydrocholesterol), and where indicated in the individual experiments, 0.6 mM phospholipid [as either egg yolk phospholipids (67.7 mole % PC and 22.7 mole % PE based on phosphorus content), phosphatidylcholine (PC), phosphatidylethanolamine (PE), dioleoyl PC, or dipalmitoyl PC], 1 mM oleic acid, and 0.5 mM monoolein. These were prepared in Hank’s balanced salts solution containing 15 mM HEPES, pH 7.4, when used in brush border studies (to preserve membrane) or in 15 mM sodium phosphate buffer, pH 7.4, containing 132 mM NaCl when used in triolein incubation studies.

Emulsions with either 1% Triton X-100 or 0.6 mM egg yolk phospholipids were prepared in 15 mM sodium phosphate buffer, pH 7.4, containing 132 mM NaCl and 200 µM [3H]cholesterol or [14C]sitosterol.

Isolation of lymph lipoproteins

Lymph collected in iced heparinized tubes containing DTNB (1 mM) was ultracentrifuged to separate the major lipoproteins by a modification of the procedure of Havel,
Eder, and Bragdon (17): chylomicrons (d < 1.006 g/ml) were collected at 16,000 g for 60 min; VLDL (d < 1.006 g/ml) at 114,600 g for 14 hr; LDL (d < 1.063 g/ml) at 114,600 g for 14 hr; and HDL (d < 1.21 g/ml) at 114,600 g for 22 hr.

Separation of chylomicron core and coat

The chylomicron nonpolar core and the polar coat were prepared by the method of Zilversmit (18). Briefly, the chylomicron preparation was frozen at -70°C. After overnight at this temperature, the tubes were slowly brought to room temperature. The freezing and thawing process was repeated three or four times until an oil phase became visible. Ten ml of distilled water was added to the preparations and the tubes were centrifuged at low speed (1500 g for 20 min). The upper layers containing the core and any intact chylomicrons and the subnatant containing the coat were collected with the aid of a tube slicer. The subnatant fraction was centrifuged at 100,000 g for 24 hr and the pellet was collected and considered to be pure coat. Corrections for cross-contamination between core and coat fractions were applied on the assumption that pure core does not contain phospholipids (18). Thus phospholipids in the core fraction were considered as contaminants arising from the coat and any undisrupted chylomicrons. Phosphorus in phospholipids (PL) was determined by the method of Rouser, Siakotos, and Fleischer (19). Esterified and free sterols were calculated separately. The calculation for each was as follows:

sterol (pure core) = sterol in core fraction -A - B

where,

A = sterol content in total subnatant fraction
\[ \times \frac{\text{volume (ml) core fraction}}{\text{volume (ml) total}} \]

B = C x sterol content \text{whole chylomicron} / PL content \text{whole chylomicron}

C = PL\text{core fraction} - [PL\text{total subnatant fraction} \times \frac{\text{volume (ml) core fraction}}{\text{volume (ml) total}}]

Preparation of brush border

Brush border membranes were prepared from the proximal half of the small intestine as described by Kessler et al. (20). These were resuspended in Hank's balanced salts solution containing 15 mM HEPES, pH 7.4, 5 mM EGTA, and 4% BSA (fatty acid-free) complexed with oleic acid (4 mol/mol) to give a final protein concentration of 2 mg/ml. The purity of this preparation has been described previously (7).

Transfer of sterols from micellar solutions to brush border membranes in vitro and in vivo

In the in vitro studies, 4 ml of the micellar solution (compositions as described in the individual experiments) were incubated with 1 ml of brush border membrane suspension (2 mg protein) at 37°C as described by Child and Kuksis (14). At the times indicated, 1- to 2-ml samples were withdrawn and released into 5 ml iced 0.9% saline containing 7 mM sodium taurocholate. This was centrifuged for 30 min at 27,000 g at 5°C. The resulting pellet was washed once in 10 ml of the same solution and recollected by centrifugation. The brush border membrane pellet was suspended in 1.0 ml distilled water, sonicated, and subjected to sterol determination by radioactivity measurement and to protein determination (21). In the in vivo studies, rats fasted overnight were administered an intragastric emulsion (1.5 ml) that contained 10 mg each of [3H]cholesterol and [14C]sitosterol. Two hours later the rats were killed and the brush border was isolated from a homogenate of mucosa scraped from the proximal half of intestine. Associated sterols were determined by radioactivity measurement.

Transfer of sterols from micellar solutions to triolein

One and one-half ml of the micellar solution (compositions as described in the individual experiments) containing either 200 μM [3H]cholesterol, [14C]sitosterol, fucosterol, campsterol, stigmasterol, cholestanol, or 7-dehydrocholesterol was incubated with 0.5 ml of triolein in a Sarstedt plastic tube (10 x 75 mm), flushed with N2 and sealed. The tubes were incubated at 37°C in an oscillating (140 oscillations/min) water bath for 6 hr, at which time transfer rate was linear. At the end of the incubation, the content of each tube was transferred to a Beckman Ultraclear centrifuge tube (2 ml), and the oil and aqueous phases were separated by centrifugation at 37°C for 1 hr at 100,000 g. The oil and aqueous phases were collected and either analyzed by counting radioactivity (cholesterol, sitosterol) with recoveries greater than 95% or by mass determination (other sterols) using GLC.

Sterol analyses

Total sterols or free and esterified sterols after separation by TLC were estimated by counting radioactivity (cholesterol, sitosterol) (22) or by mass determination (all other sterols) using GLC (5) as previously described.

RESULTS

Lymphatic absorption and lipoprotein distribution of cholesterol and sitosterol

The recoveries of total and esterified sterols in the lymph, in the lymph lipoproteins, in the core and coat fractions of the chylomicron and the recovery of total sterol in the muco-
sa 6 hr after administration of an intragastric emulsion containing 50 mg of either \(^{14}\text{C}\)cholesterol or sitosterol are summarized in Table 1. Only 0.42% of the administered sitosterol was recovered in the lymph compared to 18.2% for cholesterol (C/S ratio = 43). Of this, 11.8% of the sitosterol was esterified compared to 90% for cholesterol. Both sterols were recovered largely in the lymph chylomicrons, 70% for sitosterol and 76% for cholesterol, and to a lesser extent in VLDL, 22% for both sterols. Distribution of the two sterols in the chylomicron core versus coat differed dramatically. About 83% of the cholesterol was in the core and this was almost entirely esterified. The remainder associated with the coat was two-thirds free sterol. In contrast, 77% of the sitosterol was recovered as free sterol in the chylomicron coat. Of the 23% recovered in the core, two-thirds was esterified. Recovery of sitosterol in mucosal homogenates was about one-eleventh that of cholesterol at 6 hr.

A second study was conducted in which either 50 mg \([4-^{14}\text{C}]\)sitosterol or 1 mg \([4-^{14}\text{C}]\)cholesterol was included in the emulsions to equalize the sterol load on the intestinal mucosa and the experiment was repeated with no other modifications. The results, in Table 2, were unaffected when approximately equal mass amounts of each sterol were absorbed (173 ± 13 \(\mu\)g cholesterol, 340 ± 81 \(\mu\)g sitosterol), i.e., 17.3% of the cholesterol dose was absorbed and was 80.1% esterified compared to 68% of the sitosterol dose which was only 12.6% esterified. Lymph chylomicrons contained 85% of each sterol. Recovery of sitosterol in mucosal homogenates was again about one-eleventh that of cholesterol at 6 hr.

### Table 1. Lymphatic absorption of cholesterol and sitosterol

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol</th>
<th>Sitosterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery in lymph</td>
<td>18.2 ± 2.9</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>Esterified</td>
<td>89.8 ± 0.2</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>Lipoprotein distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicron</td>
<td>76.2 ± 3.5</td>
<td>70.1 ± 3.5</td>
</tr>
<tr>
<td>VLDL</td>
<td>22.0 ± 3.7</td>
<td>21.8 ± 2.9</td>
</tr>
<tr>
<td>LDL</td>
<td>1.1 ± 0.2</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>HDL</td>
<td>0.8 ± 0.1</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>Chylomicron distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core</td>
<td>80.2 ± 1.2</td>
<td>8.1 ± 1.6</td>
</tr>
<tr>
<td>Esterified</td>
<td>2.4 ± 0.4</td>
<td>14.9 ± 5.6</td>
</tr>
<tr>
<td>Free</td>
<td>5.8 ± 1.0</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Coat</td>
<td>11.8 ± 0.7</td>
<td>76.7 ± 6.8</td>
</tr>
<tr>
<td>Esterified</td>
<td>11.6 ± 0.9</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Lymph duct-cannulated rats were administered an intragastric emulsion (3 ml) that contained either \([4-^{14}\text{C}]\)cholesterol or \([4-^{14}\text{C}]\)sitosterol. After 6 hr, the lymph, lymph lipoproteins, and mucosa were analyzed for either total sterol or free and esterified sterol as indicated. Data are expressed as mean ± SE for \(n = 5\).

### Table 2. Lymphatic absorption of cholesterol and sitosterol

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol</th>
<th>Sitosterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery in lymph</td>
<td>17.3 ± 1.3</td>
<td>0.68 ± 0.18</td>
</tr>
<tr>
<td>Esterified</td>
<td>80.1 ± 1.3</td>
<td>12.6 ± 0.8</td>
</tr>
<tr>
<td>Lipoprotein distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicron</td>
<td>85.1 ± 1.5</td>
<td>85.0 ± 1.5</td>
</tr>
<tr>
<td>Esterified</td>
<td>81.1 ± 1.3</td>
<td>12.6 ± 0.9</td>
</tr>
<tr>
<td>Others</td>
<td>14.9 ± 1.5</td>
<td>15.0 ± 1.5</td>
</tr>
<tr>
<td>Esterified</td>
<td>75.0 ± 1.4</td>
<td>12.0 ± 0.8</td>
</tr>
<tr>
<td>Recovery with mucosa</td>
<td>30.5 ± 1.8</td>
<td>2.7 ± 0.4</td>
</tr>
</tbody>
</table>

Thoracic duct-cannulated rats were administered an intragastric emulsion (3 ml) that contained either \(1.0 \text{ mg} [4-^{14}\text{C}]\)cholesterol or 50 mg \([4-^{14}\text{C}]\)sitosterol. After 6 hr, the lymph, lymph chylomicrons, combined lymph VLDL, LDL, and HDL, and mucosa were analyzed for total sterol or free and esterified sterol as indicated. Data are expressed as mean ± SE for \(n = 4\).

### Uptake of cholesterol and sitosterol by brush border membranes in vivo

Two hours after the intragastric administration of both sterols in an emulsion, 5-fold more cholesterol than sitosterol was associated with both the brush border membranes and the homogenate from which they were prepared as shown in Table 3. The pellet fraction that contained subcellular organelles and was collected during the preparation of the brush border membranes displayed a similar cholesterol to sitosterol ratio.

### Uptake of cholesterol and sitosterol by brush border membranes in vitro

The time course of either 100 \(\mu\text{M} [^{3}\text{H}]\)cholesterol or \([^{14}\text{C}]\)sitosterol uptake by brush border membranes from a micellar solution containing sodium taurocholate and egg yolk phospholipids is shown in Fig. 2. The uptake of cholesterol was 2.5- to 3.0-fold greater at any time point.
Uptake of neither sterol occurred in the absence of bile salt or at 0°C (not shown).

Micellar composition and uptake of cholesterol and sitosterol by brush border membranes in vitro

The brush border uptake of cholesterol and sitosterol from micellar solutions containing 6.6 mM sodium taurocholate and 100 μM [3H]cholesterol or [14C]sitosterol was compared. As shown in Table 4, the uptake of cholesterol was 2.5- to 2.8-fold greater than that of sitosterol from phospholipid containing bile salt micelles and 3.5-fold greater from micelles lacking phospholipid.

Phospholipid type in the taurocholate micelle was varied and the effect on either cholesterol or sitosterol uptake by the brush border was measured. As seen in Table 5, the uptake of both sterols by the brush border was nearly twofold better from micelles containing phosphatidylcholine relative to phosphatidylethanolamine. Thus, no change was seen in the cholesterol/sitosterol ratio. In a separate experiment, brush border uptake of cholesterol was favored slightly (22.5 ± 2.0 vs. 30.0 ± 2.0 nmol/mg protein) from taurocholate micelles containing dioleoyl relative to dipalmitoyl phosphatidylcholine, while sitosterol uptake was unaffected.

Relative affinity of sterols for micellar solution

The transfer of a series of absorbable and nonabsorbable sterols from micellar solution (taurocholate, egg yolk PL) to triolein is shown in Fig. 3. Twenty-five to 40% of sterols classified as absorbable (cholesterol, campesterol, cholestanol, 7-dehydrocholesterol) transferred to the triolein in 6 hr at 37°C, while those known to be nonabsorbable (fucos-
terol, sitosterol, stigmasterol) transferred 2- to 6-fold less well. The dependence of differential sterol transfer on mixed micellar type is shown in Fig. 4. Cholesterol and sitosterol transfer from emulsions prepared with Triton X-100 or egg yolk phospholipids or from mixed taurocholate micelles of varying compositions as indicated were compared. Cholesterol and sitosterol transfer differential was apparent only lacking phospholipid. Moreover, the relative absorption differential (18.7% compared to 2.0%) was not altered when the two sterols were intraduodenally administered in the same physical state (Fig. 1), i.e., completely in micellar solution.

Both sterols appeared predominantly in the chylomicron fraction (Tables 1 and 2) isolated from lymph. Of note, however, was the difference in their distribution in the emulsified lipid meals to equalize the sterol content in the emulsified lipid meals to equalize the sterol load on the intestine (Table 2) allowed an average absorption of 173 μg of cholesterol and 340 μg of sitosterol, representing 17.3% and 0.68% absorption of the administered sterols. Moreover, the relative absorption differential (18.7% compared to 2.0%) was not altered when the two sterols were intraduodenally administered in the same physical state (Fig. 1), i.e., completely in micellar solution.

### DISCUSSION

The results of the present study confirm the discrimination between cholesterol and sitosterol for intestinal absorption as reported by others (1-3). The typical discrimination pattern was not altered by modifications in either the amount or physical form of the sterols in the lipid meal. When equal mass amounts (50 mg) of each sterol were administered (Table 1) intragastrically, 9.1 mg of cholesterol and 0.2 mg of sitosterol were recovered in the lymph (based upon radioactivity), representing 18.2% and 0.42% of the administered sterols, respectively. Adjustment of the sterol content in the emulsified lipid meals to equalize the sterol load on the intestine (Table 2) allowed an average absorption of 173 μg of cholesterol and 340 μg of sitosterol, representing 17.3% and 0.68% absorption of the administered sterols. Moreover, the relative absorption differential (18.7% compared to 2.0%) was not altered when the two sterols were intraduodenally administered in the same physical state (Fig. 1), i.e., completely in micellar solution.

### TABLE 4. Micellar composition and brush border uptake of cholesterol and sitosterol in vitro

<table>
<thead>
<tr>
<th>Micellar Solution</th>
<th>Sterol</th>
<th>0 Min</th>
<th>30 Min</th>
<th>Net</th>
<th>C/S Ratio</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC Micelle Plus</td>
<td>C</td>
<td>14.9 ± 5.4</td>
<td>48.1 ± 7.6</td>
<td>33.2 ± 2.4</td>
<td>2.8 ± 0.14</td>
<td>1.86 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>5.6 ± 1.7</td>
<td>17.5 ± 1.8</td>
<td>11.9 ± 0.8</td>
<td>0.82 ± 0.18</td>
<td>0.76 ± 0.07</td>
</tr>
<tr>
<td>MO + OA</td>
<td>C</td>
<td>136 ± 8</td>
<td>244 ± 16</td>
<td>108 ± 2.5</td>
<td>3.5 ± 0.70</td>
<td>1.46 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>115 ± 5</td>
<td>146 ± 1</td>
<td>31 ± 5</td>
<td>0.70 ± 0.07</td>
<td>1.46 ± 0.04</td>
</tr>
<tr>
<td>PL + MO + OA</td>
<td>C</td>
<td>22.5 ± 6.8</td>
<td>87.7 ± 8.0</td>
<td>65.2 ± 1.6</td>
<td>2.5 ± 0.43</td>
<td>1.46 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>14.8 ± 3.8</td>
<td>41.7 ± 2.6</td>
<td>26.9 ± 3.6</td>
<td>0.43 ± 0.14</td>
<td>1.46 ± 0.04</td>
</tr>
</tbody>
</table>

Micellar solutions (4 ml) containing taurocholate (TC), either 100 μM [3H]cholesterol (C) or [14C]sitosterol (S), and other components as indicated (PL, phospholipid; MO, monoolein; OA, oleic acid) were incubated with brush border membrane preparations (1 ml, 2 mg protein) at 37°C. Reisolated brush border membranes from aliquots taken at 0 and 30 min were analyzed for radioactivity and protein. Zero time brush border protein for PL-containing micelles was 1.68-1.98 mg and 0.82 mg for micelles lacking PL. Results are the mean ± SE for three preparations.

### TABLE 5. Micellar phospholipids and brush border uptake of cholesterol and sitosterol in vitro

<table>
<thead>
<tr>
<th>Micellar Solution</th>
<th>Sterol</th>
<th>Net Sterol Uptake</th>
<th>C/S Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC Micelle Plus</td>
<td>C</td>
<td>21.7 ± 1.9</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>7.6 ± 0.4</td>
<td>2.9</td>
</tr>
<tr>
<td>PC*</td>
<td>C</td>
<td>52.2 ± 4.1</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>21.7 ± 1.1</td>
<td>2.4</td>
</tr>
<tr>
<td>PE*</td>
<td>C</td>
<td>30.4 ± 1.6</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>11.9 ± 1.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Micellar solutions (4 ml) containing taurocholate (TC), 50 μM [3H]cholesterol or [14C]sitosterol, and the other phospholipids (PL) as indicated (PC, phosphatidylcholine; PE, phosphatidylethanolamine) were incubated with brush border membrane preparations (1 ml, 2 mg protein) at 37°C for 30 min. Reisolated brush border membranes were analyzed for sterol radioactivity and protein. The results (corrected for zero time uptake) are the mean ± SE for three or four preparations.

*Sixty eight percent PC, 23% PE.

*Purified from egg yolk phospholipid.
Fig. 3. Affinity of absorbable and nonabsorbable sterols for micellar solution. Micellar solutions (1.5 ml) containing taurocholate, egg yolk phospholipids, and 200 μM sterol, as indicated, were incubated with 0.5 ml triolein at 37°C for 6 hr. The micellar and oil phases were separated by centrifugation and analyzed for sterol content by radioactivity counting or GLC. The results represent the mean ± SE of four determinations.

Although the esterification of sitosterol is severely limited, this cannot represent a major discriminatory step since only a modest amount of sitosterol is associated with the mucosa during absorption. From a 50-mg dose (Table 1), an average of 0.55 mg of sitosterol and 5.8 mg of cholesterol (i.e., one-eleventh) were recovered with the mucosa. Differential mucosal sterol recovery was reported earlier by Borgström (13) and more recently by Ikeda and Sugano (15). As a consequence, major discrimination between sterols for absorption must occur extracellularly.

In support of this, the present data demonstrate a differential affinity of absorbable and nonabsorbable sterols for the bile salt micelle and leave open, as well, the possibility for differential sterol uptake by the brush border membrane. In consideration of the former, the transfer to triolein (sterol-free) of a series of absorbable and nonabsorbable sterols solubilized in bile salt micelles was compared (Fig. 3). Absorbable sterols transferred at two to six times the rate of nonabsorbable sterols. For example, after 6 hr at 37°C while sterol transfer was still linear with time, 40% of the cholesterol and 7-dehydrocholesterol transferred while 10% or less of sitosterol and stigmasterol transferred. About 25% of the campesterol, reported to have intermediate absorbability (4), transferred. Transfer differential was

Fig. 4. Micellar composition and affinity for cholesterol and sitosterol. Micellar solutions (1.5 ml) containing taurocholate, varying components as indicated, and either 200 μM [3H]cholesterol or [14C]sitosterol were incubated with 0.5 ml triolein at 37°C for 6 hr. As controls, emulsions (1.5 ml) prepared with Triton X-100 or egg yolk phospholipids and containing 200 μM sterols were similarly incubated. Phases were separated by centrifugation and analyzed for sterol radioactivity. Results are the mean of two determinations.
lyzed for sterol radioactivity. Results are the mean determinations for the taurine conjugates. The glycine conjugates gave olein at 37°C for 6 hr. Phases were separated by centrifugation and analyzed for sterol radioactivity. Results are the mean ± SE of four determinations for the taurine conjugates. The glycine conjugates gave similar results which are not shown.

Fig. 5. Micellar bile salts and affinity for cholesterol and sitosterol. Micellar solutions (1.5 ml) containing egg yolk phospholipids, the taurine or glycine conjugates of cholate, chenodeoxycholate, or deoxycholate, and 200 μM [14C]sitosterol were incubated with 0.5 ml triolein at 37°C for 6 hr. Phases were separated by centrifugation and analyzed for sterol radioactivity. Results are the mean ± SE of four determinations for the taurine conjugates. The glycine conjugates gave similar results which are not shown.

dependent upon sterol solubilization in bile salt micelles (Fig. 4). The differential between cholesterol and sitosterol transfer was affected significantly by the micellar bile salt type with the greatest transfer differential obtained with the taurine or glycine conjugates of cholate and the least with the same conjugates of deoxycholate (Fig. 5). Based upon the Armstrong and Carey (16) report, the observed large transfer differential from taurocholate micelles and the narrowing of this differential from dihydroxy bile salt micelles would have been predicted. Moreover, as observed, both sterols would have been predicted to transfer at a greater rate from the dihydroxy bile salt micelles for which they have lesser affinity (16). The slightly greater sterol transfer differential observed with the taurodeoxycholate relative to the taurochenodeoxycholate micelle based upon their relative hydrophilic nature was not predicted nor easily explained. The addition of monoolein and oleic acid to the taurocholate micelle did not affect the transfer differential (Fig. 4). However, the absence of phospholipid from this mixed micelle increased the rate of transfer of both sterols several fold while maintaining a substantial differential. Thus, phospholipid increases sterol affinity for the taurocholate-containing micelles. If these in vitro studies have applicability in vivo as we hypothesize, then differential sterol affinity for the bile salt micelle which influences the rate of sterol transfer plays a major role in sterol discrimination for absorption.

In addition to differential sterol affinity for the micelle, evidence for differential uptake of cholesterol and sitosterol at the brush border membrane was obtained both in vivo and in vitro. In the in vivo studies in which mass amounts (Table 3) of both sterols were administered, the cholesterol to sitosterol ratio in the brush border was 5 to 1. This approximates the ratio reported by Child and Kuksis (14) in their in vitro brush border binding studies conducted with 7-dehydro sterols. This ratio was duplicated in the whole mucosal homogenate and was only slightly greater in the pellet containing subcellular organelles. Our observation (data not shown) and that of Child and Kuksis (23) that selectivity at the brush border surface disappears when sterols are presented in emulsion form suggest that the selectivity is related to uptake through the membrane rather than to a surface phenomenon.

The in vitro studies (Fig. 2) revealed a time-dependent uptake of each sterol from micellar solution with cholesterol uptake favored by a ratio approaching 3 to 1. The ratio differential increased when phospholipid was omitted from the micelle. Under this condition, however, membrane protein was solubilized (Table 4) as reported earlier by Child and Kuksis (24) and adsorption of sterols at zero time was much higher than when phospholipid was included. For these reasons, the accuracy of the ratio is in question. In fact, Child and Kuksis (23) reported no sterol selectivity by intact rat jejunal villus cells when micelles lacked phospholipid. However, in our studies when phospholipid was a component of the bile salt micelle, other micellar components (MO, OA, Table 4) and the type of phospholipid (egg yolk, PC, or PE) did not affect in any major way the relative uptake of the two sterols (Table 5). This observation also contrasts with that of Child and Kuksis (23) who observed an increase in the differential in favor of cholesterol uptake as phosphatidylethanolamine was increased in the micelle. We cannot account for these differences other than to note that the present studies were conducted with brush border membranes and those that are in contrast were conducted in villus cells, which have three membrane surfaces not representative of the brush border.

Based upon the present data, the discrimination between cholesterol and sitosterol for intestinal absorption may be accounted for by the combination of two major discriminatory events, i.e., the greater uptake of cholesterol by the brush border membrane and the greater rate of cholesterol delivery from the luminal bile salt (cholate) micelle to the membrane. However, it is possible that what appears to be brush border discrimination between sterols could be accounted for predominantly by preferential delivery of cholesterol. In vivo studies in bile-fistula rats receiving "artificial" bile containing micellar taurochenodeoxycholate from which cholesterol and sitosterol transfer equally in vitro (Fig. 5) should aid in distinguishing between the two events.
An effort to assess the relative quantitative importance of the identified discriminatory events is complicated since the data are drawn from a combination of in vitro and in vivo studies (clearly not equivalent). However, when we consider the 1:1 cholesterol to sitosterol ratio in lymph that was obtained under conditions where the two sterols were co-administered and fully in micellar solution, then the differential transfer of sterols from the bile salt micelle (4:1, Figs. 3 and 4) and the differential uptake of sterols (5:1, Table 3) with minor input from the intracellular discrimination between sterols for esterification could account totally for the observed discrimination.

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**REFERENCES**