# Postnatal development of intestinal bile salt transport. Relationship to membrane physico-chemical changes

James E. Heubi[1] and Janette L. Fellows

Division of Gastroenterology and the General Clinical Research Center, Children's Hospital Research Foundation, Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229

**Abstract** The postnatal development of intestinal bile salt transport in the rat was examined using the villus technique. Jejunal uptake of taurocholate was linear with respect to incubation concentration at all study ages. Ileal uptake was linear with taurocholate concentration during the first 2 postnatal weeks; a curvilinear relationship indicating the presence of saturable transport appeared during the third week. With the appearance of ileal active transport at age 3 weeks, the $K_m$ (app) was constant at 0.49 mM, 0.59 mM, and 0.50 mM in 3-week, 4-week, and adult animals, respectively. The V(app) was 14.65 nmol $\cdot$ mg$^{-1}$ (dry wt) $\cdot$ min$^{-1}$ at 3 weeks and declined with age to 11.40 and 10.51 nmol $\cdot$ mg$^{-1}$ (dry wt) $\cdot$ min$^{-1}$ in 4-week and adult animals, respectively. The role of physico-chemical changes in the microvillus membrane in the development of ileal active transport was examined. With increasing postnatal age, microvillus membrane cholesterol content rose while the phospholipid content remained unchanged in both ileum and jejunum. Corresponding rises in the cholesterol/phospholipid ratio were observed in both sites. Simultaneously, the microvillus membrane fatty acid composition was changing from predominantly saturated to unsaturated species in both ileum and jejunum. The microvillus membrane fluorescence anisotropy (r) increased with postnatal age in jejunum when measured at 25°C and 37°C and ileum when measured at 25°C; however, no change was noted in ileum when measured at 37°C. Ileal active bile salt transport develops during the third postnatal week, and is associated with concurrent changes in membrane lipid composition and fluidity when measured at 25°C. ■■ Although these studies suggest a possible relationship between postnatal changes in membrane fluidity and the development of ileal active bile salt transport, the absence of fluidity change at 37°C suggests the findings may not be physiologically significant. — **Heubi, J. E., and J. L. Fellows.** Postnatal development of intestinal bile salt transport. Relationship to membrane physico-chemical changes. *J. Lipid Res.* 1985. **26:** 797–805.

**Supplementary key words** ontogeny $\cdot$ taurocholate $\bullet$ cholesterol $\bullet$ phospholipid $\bullet$ fluorescence anisotropy $\bullet$ membrane fluidity

Mature mammalian species including man have a highly efficient enterohepatic circulation of bile salts. With each of the six to nine daily meal-stimulated cycles, 95–97% of the secreted bile salts are reabsorbed from the gastrointestinal tract (1, 2). Passive diffusion of bile salts along the entire length of the small and large intestine and an active transport system localized to the terminal ileum are responsible for this efficient absorption (3–5). Immature species including pre-term and term human infants have reduced bile salt pools and less efficient intestinal and hepatic transport of bile salts compared to adults (6–11). Previous studies have suggested that in rats, dogs, guinea pigs, and man ileal active transport either is absent or severely compromised at birth and maturation of this transport system transpires at a variable, species-dependent rate (11–14).

The relationship between the membrane physico-chemical state and function, specifically relating to enzyme and transport systems, has been characterized in several organs and species (15–17). However, alterations in membrane lipid composition, fluidity, and their relationships to the perinatal development of enzyme and transport systems have been examined on only a limited basis (18–21).

The present study was designed to examine the development of intestinal bile salt transport in the suckling, weanling, and adult rat. We also sought to relate the changes in membrane lipid composition and fluorescence anisotropy to the development of ileal active bile salt transport. The results suggest that ileal active bile salt transport develops during the third postnatal week coincident with changes in microvillus membrane lipid composition and fluidity.

---

## MATERIALS AND METHODS

### Animals

Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals (aged 1, 2, or 3 weeks) were obtained from litters of dated pregnant dams delivered in our animal facility or directly from Charles River laboratories with a 2–3 day period of adaptation before study (4-week and 6–8-week (adults)). Animals were maintained in our animal facility with diurnal light cycling and allowed to suckle (1-, 2-, 3-week) or eat Purina rat chow (4-week and adult) ad libitum and water until the time of study. All animals were studied in the fed state, weighed, and anesthetized with diethyl ether prior to study.

### Intestinal bile salt transport

The villus incubation technique was used as previously described (14). Segments of jejunum and ileum weighing 5–15 mg were pooled in separate holding baths containing Krebs-Ringer bicarbonate buffer, pH 7.4, with 10 mM glucose and oxygenated with 95% $O_2$–5% $CO_2$. After all pieces were excised, they were transferred to individual gassed incubation flasks and incubated with Krebs buffer with 10 mM glucose containing 0.1, 0.25, 0.5, 1.0, 2.0, or 3.5 mM sodium taurocholate (Calbiochem, La Jolla, CA; purity 96% by manufacturer) and tracer [24-$^{14}$C]taurocholic acid (New England Nuclear, Boston, MA; sp act 52.0 mCi/mmol; 98% pure by manufacturer and by thin-layer chormatography in our laboratory). Segments were incubated with shaking at 140 cpm for 2 min and the flask contents were then poured through a nylon mesh screen where the tissue was trapped. The tissue was rinsed, placed in a vial containing Krebs buffer, and quick-frozen in a dry ice–acetone bath. The vial contents were lyophilized overnight. The dried tissue was tapped free of freeze-dried salts and weighed. The villi were chipped free from the underlying crypt, submucosal, and muscular layers. The desiccated villi were dissolved in 1.0 ml of Soluene-100 (Packard Instruments, Downers Grove, IL) at 60°C, 0.6 ml 15% w/w ascorbic acid (Eastman Kodak Co., Rochester, NY), and 10.0 ml scintillation cocktail (Aquassure, New England Nuclear) and radioactivity was measured. Previous experiments demonstrated adherent extracellular fluid to be negligible following lyophilization; tissue viability persisted for at least 12 min and uptake was linear with time from 0 to 135 sec (14).

### Membrane isolation

Microvillus membrane was prepared by the calcium precipitation technique of Schmitz et al. (21) as modified by Kessler et al. (22). After the animals were anesthetized with diethyl ether, the entire small intestine was removed from the ligament of Trietz to the cecum. The gut was divided into quarters and the proximal fourth was taken for jejunal studies and the distal fourth for ileal studies. Membranes were pooled from entire litters from 1-, 2-, and 3-week animals and from four animals at 4-weeks and adults. The segments were flushed with 0.9% NaCl and thereafter scraped and suspended in 50 mM mannitol–2 mM Tris/HCl, pH 7.1. The intestine was homogenized for 2 min in a Sorval Omnimixer (Dupont Instruments, Newtown, CT). $CaCl_2$ was added to a final concentration of 10 mM, and after standing 15 min at 4°C, the homogenate was centrifuged at 7500 $g$ for 15 min. The resultant pellet was discarded and the supernatant was centrifuged at 26,000 $g$ for 40 min. The pellet was homogenized at low speed in a Kimas glass-Teflon homogenizer in 20 ml of 400 mM mannitol/2 mM HEPES/Tris, pH 7.4. The homogenate was centrifuged at 28,000 $g$ for 30 min and the pellet was resuspended in 1–3 ml of 5 mM Tris/HCl, pH 8. Microvillus membrane purity was verified at all study ages using transmission electron microscopy which showed vesicles of various shapes and absence of other organelles. Marker enzymes were examined in the homogenate and in the purified microvillus membranes by methods described below. Alkaline phosphatase was enriched 10.5 ± 1.6-fold (mean ± SE) and sucrase, 13.5 ± 3.4-fold. In selected preparations, lactase enrichment was comparable at all ages (8 to 12-fold).

### Lipid compositional and fluorescence anisotropy studies

After lipid extraction by the method of Folch, Lees, and Sloane Stanley (23), cholesterol was measured by a coupled enzymatic assay using a buffer solution containing catalase, cholesterol esterase, and cholesterol oxidase (Boehringer, Indianapolis, IN) (24), and phospholipid was measured as total lipid phosphorus by the method of Bartlett (25). Both cholesterol and phospholipid concentrations were divided by tissue protein content (see below) and expressed as $\mu$mol/mg protein. Cholesterol/phospholipid ratios were expressed as the molar concentration of cholesterol divided by phospholipid. Fatty acids were measured by gas–liquid chromatography after saponification of lipids in methanolic KOH, extraction, and methylation using the method of MacGee and Allen (26). The percentage composition of methyl esters was quantitated using a Hewlett-Packard 5710A gas–liquid chromatograph equipped with a 3380A Integrator (Hewlett-Packard, Avondale, PA). Fluorescence anisotropy was determined according to the method of Shinitzky and Barenholz (27) utilizing the lipid-soluble fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co., Milwaukee, WI). Membrane samples were suspended in 5 mM Tris-HCl buffer which contained 300 $\mu$g of protein to minimize probe–probe interaction. All

samples were sonicated prior to fluorescence measurements and the measured anisotropy (r) was calculated,

$$r = \frac{I_\| - I_\perp \ (1)}{I_\| + 2I_\perp}$$  *Eq. 1)*

where $I_\|$ and $I_\perp$ are relative fluorescence intensities parallel and perpendicular, respectively, to the direction of the excitation beam. Measurements were made at 37.0 ± 0.1°C ($\bar{x}$ ± SD) and 25.0 ± 0.2°C ($\bar{x}$ ± SD) using a Perkin-Elmer fluorescence spectrophotometer Model 650-10S (Perkin Elmer, Norwalk, CT) equipped with a polarizing filter. Measurements were made at an excitation wavelength of 366 nm and an emission wavelength of 430 nm.

## Other measurements

Protein was measured by the method of Lowry et al. (28) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. Sucrase was measured by the method of Dahlqvist (29) using the coupled enzyme reaction of peroxidase–glucose oxidase (Sigma Chemical Co.) (29). Alkaline phosphatase was measured by estimating production of $p$-nitrophenol liberated from $p$-nitrophenol phosphate at 420 nm using a recording spectrophotometer over 15 min (30, 31).

## Calculations

All results are expressed as mean ± SD except $K_m$ and V(app) from the computer analysis described below in which derived values are mean ± SE. Bile salt uptake (J) was calculated using the equation:

$$J \ (nmol \cdot min^{-1} \cdot mg \ dry \ wt^{-1})$$
$$= \frac{cpm}{sp \ act} \div time \ (min) \div dry \ wt \ (mg).$$  *Eq. 2)*

The jejunal apparent monomer permeability coefficient, P(app), is characterized by the equation:

P(app) $(nmol \cdot min^{-1} \cdot mg \ dry \ wt^{-1} \cdot mM^{-1})$ = J/C  *Eq. 3)*

where C equals the taurocholate monomer concentration. The apparent maximal transport velocity (V(app), nmol $\cdot min^{-1} \cdot mg \ dry \ wt^{-1}$) and the apparent Michaelis-Menten constant ($K_m$(app), mM) was calculated by means of a computer program using CLINFO (Division Research Resources, National Institutes of Health) that permitted a weighted least-squares fit of individual data points (32). Because of animal-size limitations, a single kinetic curve for each animal could not be constructed and results were pooled. Age-related differences in $K_m$(app) and V(app) differences were tested for significance using the $Z$-test (33). All other comparisons of measurements between different age animals were made using analysis of variance (ANOVA). If statistical sigificance was observed, further analysis to determine differences between age groups was performed using Newman-Keuls multiple range testing.

## RESULTS

### Intestinal taurocholate uptake

At each study age, jejunal uptake rates for taurocholate were linear with increasing concentration (**Fig. 1**). A significant increase ($P < 0.001$) in P(app) was observed with increasing age; however, only the P(app) value for adults differed significantly from all other ages ($P \le 0.05$). Ileal taurocholate transport was linear with respect to taurocholate concentration in 1- and 2-week rats, a time when ileal and jejunal uptake rates were comparable (Fig. 1a,b). A curvilinear relationship between ileal uptake and taurocholate concentration appeared at 3 weeks and persisted with increasing age. Similar $K_m$(app) values were observed for 3-week, 4-week, and adult rats; however, a significant decline of V(app) was observed from a peak of 14.65 nmol $\cdot min^{-1} \cdot mg \ dry \ wt^{-1}$ at 3 weeks to a plateau in 4-week and adult animals (11.40 and 10.51 nmol $\cdot min^{-1} \cdot mg \ dry \ wt^{-1}$, respectively) (**Table 1**).

### Microvillus membrane lipid composition and fluorescence anisotropy

Ileal microvillus membrane cholesterol content increased with increasing age (**Table 2**); however, the change did not reach statistical significance ($P = 0.11$). Jejunal cholesterol content increased significantly ($P < 0.001$) with age. The cholesterol content of membrane from 1-week, 2-week, and 3-week rats was significantly lower than that of adults, but no differences were noted between content from membranes from 1-week and 2-week animals and any other study ages. The cholesterol content of jejunal microvillus membrane from 2-week-old rats was also significantly lower than that of 4-week-old rats. Microvillus membrane phospholipid content was remarkably constant with age (Table 2). No significant changes in phospholipid content with age were observed in either ileum or jejunum. The cholesterol/phospholipid ratio (C/PL) increased significantly (both $P < 0.001$) with age in both ileum and jejunum. From age 1-week to adult there was an approximate twofold increase in C/PL in jejunum and ileum. The ileal C/PL was significantly higher in 4-week-old rats and adults compared to all younger age groups; however, there was no significant difference in C/PL between 4-week-old rats and adults. In the jejunum, there were no differences in the membrane C/PL between 1-week, 2-week, 3-week, and 4-week animals; however, adult microvillus membrane C/PL differed significantly from 1-week, 2-week, 3-week, and 4-week animals.

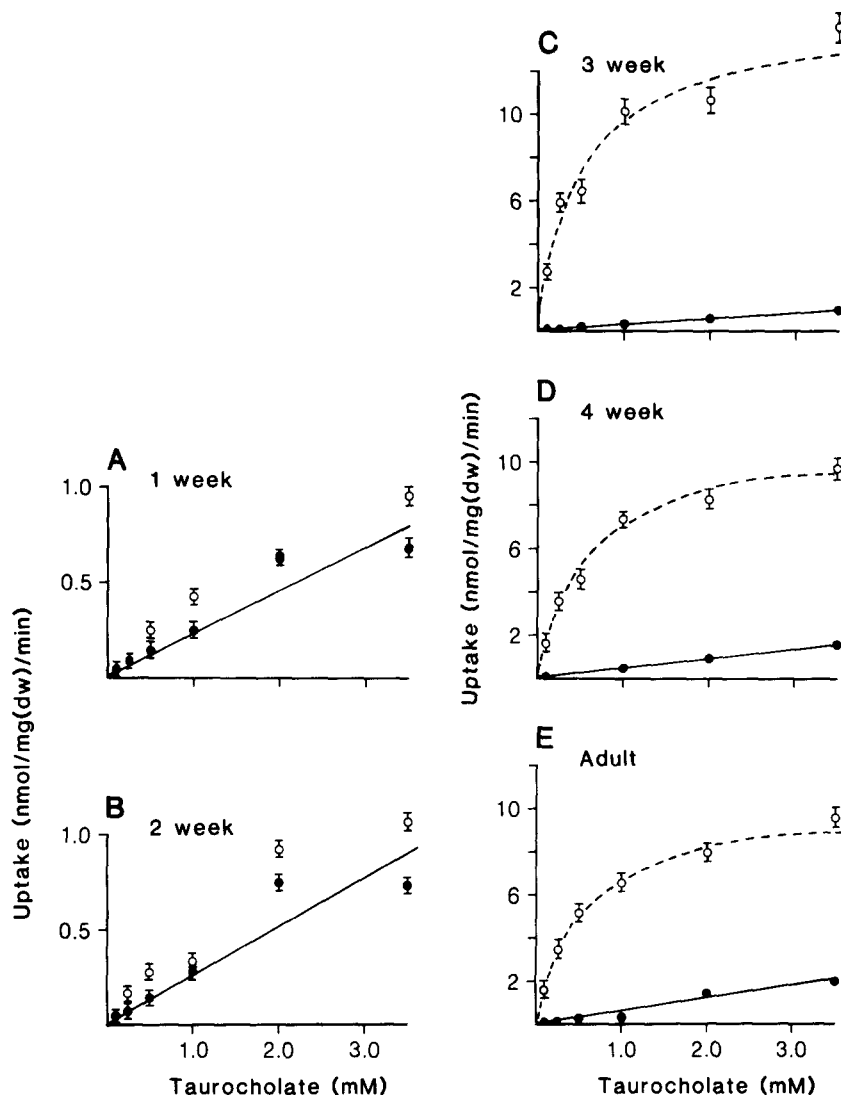Microvillus membrane fatty acid composition changed with age in both ileum and jejunum (**Table 3**). Palmitic

**Fig. 1.** Age-related changes in intestinal taurocholate uptake. Relation between incubation concentration and uptake of taurocholate in jejunum (solid lines) and ileum (dashed lines) from A, 1-week; B, 2-week; C, 3-week; D, 4-week; and E, adult rats. Values are means ± SE and represent 12–18 determinations. Best fit lines depicting ileal uptake from 3-week, 4-week, and adult rats were generated using a computer program that permitted a weighted least-squares fit of data points (ref. 32).

(16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and arachadonic acids (20:4) predominated in ileum and jejunum at all ages. Significant differences between the findings in each age group were noted for the proportion of 18:1 ($P = 0.006$), 18:2 ($P < 0.001$), 20:3 ($P < 0.001$), and 20:4 ($P < 0.001$) fatty acids in the jejunum and 18:0 ($P < 0.001$), 18:1 ($P < 0.001$), 18:2 ($P < 0.001$), and 20:4 ($P = 0.003$) in the ileum. There were no obvious shifts toward larger proportions of longer or shorter chain length fatty acids with age. The proportion of saturated fatty acids in microvillus membrane declined significantly with increasing age in both ileum ($P = 0.001$) and jejunum ($P = 0.008$). In the ileum, the saturated to unsaturated fatty acid (S/U) ratio declined from 1.74 at

1-week to 0.90 in adults, whereas in the jejunum it declined from 1.05 at 1-week to 0.76 in adults. Associated with generalized trends toward increased proportions of unsaturated fatty acids in ileum with increasing age, significant changes were noted between ileum from 1-week-old rats compared to 3-week, 4-week, and adults and 2-week old rats compared to 4-week and adults (Table 3). Differences between proportions of unsaturated fatty acids in jejunum could only be detected between adult and 1- and 2-week-old rats and 4-week-old and 2-week-old animals.

Fluorescence anisotropy (r) measurements at 25°C and 37°C for ileal and jejunal microvillus membranes are shown in **Table 4.** No significant differences in fluores-

TABLE 1. Apparent jejunal permeability coefficients and kinetic characteristics of ileal active transport

| Age | Number of Animals | Jejunum | Ileum | |
|-----|-------------------|---------|-------|---|
| | | P(app) | $K_m$ (app) | V (app) |
| | | $nmol \cdot min^{-1} \cdot mg^{-1} \cdot mM^{-1}$ | $mM$ | $nmol \cdot min^{-1} \cdot mg^{-1}$ |
| 1-Week | 18 | $0.265 \pm 0.075^{a,b}$ | 0 | 0 |
| 2-Week | 26 | $0.273 \pm 0.083^{a,b}$ | 0 | 0 |
| 3-Week | 19 | $0.224 \pm 0.014^{a,b}$ | $0.49 \pm 0.05^a$ | $14.65 \pm 0.52^c$ |
| 4-Week | 15 | $0.269 \pm 0.016^{a,b}$ | $0.59 \pm 0.06^a$ | $11.40 \pm 0.35^a$ |
| Adult | 15 | $0.435 \pm 0.020$ | $0.50 \pm 0.05^a$ | $10.51 \pm 0.32^a$ |

Passive permeability coefficients, P(app), measured at concentrations at which taurocholate would be in monomeric states. All values are means ± SD.

[a] $P$ not significant between indicated ages.

[b] $P \leq 0.05$ compared to adult.

[c] $P < 0.001$ compared to ages 4-week and adult.

cence anisotropy were noted in ileum with changing age at 37°C; however, fluorescence anisotropy increased significantly ($P < 0.001$) with increasing age when measured at 25°C. Significant differences ($P \leq 0.05$) were noted between fluorescence anisotropy in microvillus membrane from 1- and 2-week-old rats when compared to 3-week, 4-week, and adult animals. Jejunal anisotropy increased significantly ($P < 0.001$) with age when measured at both 25°C and 37°C. A pattern similar to that observed in the ileum at 25°C was found in the jejunum when measurements were made at both 25°C and 37°C (Table 4). At all ages except 4-weeks, ileal microvillus membrane fluorescence anisotropy was significantly higher than jejunum when measured at 37°C. Ileal microvillus membrane fluorescence anisotropy was higher than jejunal at all ages except 3-weeks ($P = 0.087$) and 4-weeks ($P = 0.076$) when measured at 25°C.

### Sucrase activity

Sucrase activity could not be measured in microvillus membrane obtained from either the ileum or the jejunum of 1-week-old rats. With increasing age, jejunal and ileal sucrase activities rose. Jejunal activity increased sig-

nificantly to age 3-weeks (ileum, $399.7 \pm 247.5$ $\mu$mol/g of protein·min; jejunum $611.5 \pm 280.4$ $\mu$mol/g of protein ·min) after which no further change was noted. Ileal activity similarly increased at age 3-weeks after which no change was noted. Although jejunal sucrase tended to be higher than ileal, no significant differences were noted at any age except 2-weeks ($P < 0.05$).

### DISCUSSION

In all mammalian species studied to date, ileal active bile salt transport is absent at birth and develops during the first postnatal weeks (11–14). The temporal sequence of development appears to be species-dependent. In the present studies, we have shown that the rat develops ileal active taurocholate transport during the third postnatal week. When active transport was first observed at 3 weeks, the $K_m$(app) was comparable to that of older animals; however, the V(app) was modestly higher initially and declined at older ages. In our previous studies in the guinea pig, using the same technique, the $K_m$(app) was also constant after development of active transport but the

TABLE 2. Microvillus membrane lipid composition

| Age | Ileum | | | Jejunum | | |
|-----|-------|---|---|---------|---|---|
| | Cholesterol | Phospholipid | Cholesterol/ Phospholipid | Cholesterol | Phospholipid | Cholesterol/ Phospholipid |
| 1-Week | $.249 \pm .102$ (8) | $.713 \pm .315$ (7) | $.307 \pm .113^{a,b}$ (6) | $.232 \pm .113^b$ (6) | $.590 \pm .354$ (7) | $.422 \pm .146^b$ (4) |
| 2-Week | $.292 \pm .144$ (9) | $.885 \pm .215$ (6) | $.346 \pm .092^{a,b}$ (5) | $.201 \pm .085^{a,b}$ (10) | $.677 \pm .213$ (6) | $.328 \pm .072^b$ (5) |
| 3-Week | $.326 \pm .158$ (10) | $.802 \pm .294$ (9) | $.452 \pm .172^{a,b}$ (8) | $.279 \pm .089^b$ (11) | $.772 \pm .276$ (9) | $.406 \pm .102^b$ (9) |
| 4-Week | $.405 \pm .073$ (6) | $.640 \pm .270$ (4) | $.821 \pm .374$ (4) | $.354 \pm .054$ (6) | $.760 \pm .294$ (4) | $.519 \pm .202^b$ (4) |
| Adult | $.397 \pm .081$ (6) | $.616 \pm .259$ (7) | $.761 \pm .201$ (5) | $.468 \pm .162$ (6) | $.618 \pm .291$ (7) | $.939 \pm .253$ (5) |

Number of determinations in parentheses. Each determination for ages 1-, 2-, and 3-weeks represents pools of animals from one litter, and determinations for ages 4-weeks and adult represent pools of four animals. All cholesterol and phospholipid results are expressed as $\mu$mol/mg protein. Cholesterol/phospholipid values are molar ratios. All values are means ± SD.

[a] $P \leq 0.05$ versus 4-week.

[b] $P \leq 0.05$ versus adult.

TABLE 3. Fatty acid composition of microvillus membrane

| Group | Segment | Myristic 14:0 | Palmitic 16:0 | Palmitoleic 16:1 | Stearic 18:0 | Oleic 18:1 | Linoleic 18:2 | 20:3 | Arachadonic 20:4 | Total Saturated | Total Unsaturated |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-Week | Jejunum (9) | 2.7 ± 0.27 | 25.6 ± 1.8 | ND | 23.1 ± 3.9 | 19.5 ± 2.1 | 16.3 ± 2.4[a] | 1.1 ± 0.6 | 9.5 ± 2.4[a,c] | 51.3 ± 6.6[c] | 48.7 ± 6.6[c] |
| | Ileum (7) | 4.5 ± 4.5 | 26.3 ± 3.4 | ND | 33.4 ± 5.3[b,c] | 10.8 ± 2.1[b,c] | 7.9 ± 1.3[a,b,c] | ND | 15.7 ± 7.7[a,c] | 63.9 ± 8.5[a,b,c] | 36.1 ± 8.5[a,b,c] |
| 2-Week | Jejunum (10) | 3.0 ± 2.5 | 24.9 ± 5.0 | 0.8 ± 1.3 | 22.9 ± 3.2 | 16.8 ± 0.6[c] | 17.1 ± 2.5[a] | 1.9 ± 2.2 | 9.8 ± 1.9[a,c] | 50.8 ± 4.4[c] | 49.2 ± 4.4[c] |
| | Ileum (9) | 1.0 ± 1.5 | 24.8 ± 3.6 | 1.6 ± 2.1 | 32.3 ± 3.0[b,c] | 10.5 ± 2.4[b,c] | 10.4 ± 2.7[a] | 1.4 ± 1.5 | 16.2 ± 4.8[c] | 58.1 ± 5.7[b,c] | 41.9 ± 5.7[b,c] |
| 3-Week | Jejunum (9) | 2.7 ± 1.2 | 24.5 ± 2.4 | 0.4 ± 0.9 | 21.6 ± 3.9 | 18.2 ± 2.1 | 22.6 ± 2.7 | 6.1 ± 1.2[b,c,d] | 6.1 ± 1.2 | 49.0 ± 4.2 | 51.0 ± 4.2 |
| | Ileum (10) | 0.8 ± 0.9 | 20.8 ± 4.7 | 0.5 ± 0.9 | 29.6 ± 4.7 | 17.2 ± 3.8 | 14.1 ± 1.9 | 2.5 ± 1.3 | 10.3 ± 2.2 | 51.2 ± 8.2 | 48.7 ± 8.2 |
| 4-Week | Jejunum (4) | 0.5 ± 0.8 | 23.3 ± 3.6 | 1.8 ± 1.2 | 20.9 ± 0.6 | 17.4 ± 1.8 | 20.3 ± 0.2 | 1.3 ± 0.2 | 7.4 ± 0.6 | 44.5 ± 3.8 | 55.5 ± 3.8 |
| | Ileum (5) | ND | 25.2 ± 7.4 | 0.6 ± 1.3 | 22.7 ± 1.6 | 20.3 ± 2.0 | 11.8 ± 2.2 | 1.6 ± 1.1 | 9.7 ± 1.6 | 47.0 ± 10.5 | 53.0 ± 10.5 |
| Adult | Jejunum (6) | 0.4 ± 0.5 | 20.6 ± 1.2 | 2.7 ± 1.0 | 22.0 ± 1.5 | 21.2 ± 2.0 | 19.5 ± 4.9 | 1.0 ± 0.7 | 6.2 ± 1.7 | 43.1 ± 2.4 | 56.9 ± 2.4 |
| | Ileum (6) | 0.4 ± 0.5 | 22.8 ± 1.5 | 2.2 ± 1.5 | 24.3 ± 2.2 | 20.0 ± 1.2 | 12.4 ± 4.4 | 1.7 ± 0.5 | 8.2 ± 2.2 | 47.5 ± 2.9 | 52.5 ± 2.9 |

Values represent percentage distribution. All data are expressed as means ± SD. Numbers of determinations are in parentheses. Each determination for ages 1, 2, and 3 weeks represents pools of animals from one litter and determinations for ages 4 weeks and adult represent pools of four animals. ND, not detectable.

[a] $P \leq 0.05$ versus 3-week.
[b] $P \leq 0.05$ versus 4-week.
[c] $P \leq 0.05$ versus adult.
[d] $P \leq 0.05$ versus 1-week and 2-week.

V(app) rose with increasing age (14). Only a limited number of ages (3-week, 4-week, and adult) were studied in the rat after development of ileal active transport in the present studies; however, additional studies have shown that the V(app) is at a peak during the third week in the rat (J. E. Heubi, unpublished observation), at the time of appearance of active transport. Additional work in our laboratories has shown the development of active transport in ileal brush border vesicles during the third postnatal week (Moyer, M. S., et al., submitted for publication). These findings sustain the concept that the postnatal development of the transport system in mammals is characterized by an unchanging affinity for taurocholate and changes in the numbers of binding sites as a function of age until a plateau is reached in the mature animal.

The rate of jejunal passive diffusion of taurocholate is variable during suckling, weaning, and adulthood. The passive permeability coefficient was similar at all study ages except adult. This differs from previous observations in the guinea pig in which there was a gradual decline in permeability with increasing age, which, by additional experiments, was shown to be of minimal biologic significance (14). Since only modest changes in permeability in the rat were observed, these findings suggest that no biologically significant change in the permeability of the rat jejunum to bile salts occurs with increasing age. These data oppose the idea that, during the phases of development when ileal active transport is absent, compensatory increases in jejunal permeability may "short circuit" the enterohepatic circulation and lead to improved conservation of the bile salt pool.

The factors that may modulate the postnatal development of intestinal enzyme and transport systems are incompletely understood (34, 35). The relationship between physico-chemical changes in the microvillus membrane and the development of either disaccharidase activity or ileal active bile salt transport have not been examined. Recent studies have demonstrated developmental changes in heart and liver plasma membrane and intestinal microvillus membrane composition and microviscosity (18, 19, 36). The relationships between these perinatal membrane changes and the expression of enzyme and transport systems appear less clear.

Significant postnatal changes in rat microvillus membrane lipid composition and fluidity were observed in the present study. With increasing postnatal age, the cholesterol content rose while the phospholipid content was relatively constant in both ileum and jejunum. Concurrently, the fatty acid composition changed from predominantly saturated to unsaturated species to varying degrees in both ileum and jejunum. Membrane fluidity (measured at 25°C) decreased in both ileum and jejunum with increasing postnatal age. Although it would be attractive to propose that the above changes were all developmental, it is not possible to exclude the role that

TABLE 4. Microvillus membrane fluorescence anisotropy (r) in ileum and jejunum

| | Ileum | | Jejunum | |
| --- | --- | --- | --- | --- |
| Age | 25°C | 37°C | 25°C | 37°C |
| 1-Week | .254 ± .013 (5) | .244 ± .008 (8) | .224 ± .006 (4) | .200 ± .011 (8) |
| 2-Week | .255 ± .007 (6) | .243 ± .008 (8) | .240 ± .010 (6) | .211 ± .011 (8) |
| 3-Week | .272 ± .010[a,b] (6) | .240 ± .010 (11) | .263 ± .007[a,b] (6) | .228 ± .010[a,b] (11) |
| 4-Week | .274 ± .006[a,b] (4) | .240 ± .012 (6) | .266 ± .006[a,b] (4) | .231 ± .007[a,b] (6) |
| Adult | .276 ± .008[a,b] (4) | .241 ± .007 (5) | .256 ± .012[a,b] (6) | .227 ± .009[a,b] (5) |

All values are expressed as means ± SD. Number of determinations (from pools of animals as described in Tables 2 and 3) is in parentheses.
[a] $P \leq 0.05$ versus 1-week.
[b] $P \leq 0.05$ versus 2-week.

dietary differences between suckled and chow-fed rats may play on microvillus membrane lipid composition and fluidity (37, 38).

With increasing postnatal age, the cholesterol/phospholipid molar ratio increased in both ileum and jejunum. In model membranes and biologic membranes, membrane fluidity varies inversely with the cholesterol/phospholipid molar ratio (17, 39–41). As might be predicted by a rise in the cholesterol/phospholipid molar ratio in our studies, microvillus membrane fluidity declined with increasing age in jejunum (when measured at 25°C and 37°C) and ileum (when measured at 25°C). Coincident with the rise in the cholesterol/phospholipid molar ratio, the membrane fatty acid composition changed. In both ileum and jejunum, a change from predominantly saturated fatty acids during suckling to unsaturated fatty acids during weaning and adulthood was observed. Reductions in phospholipid and total membrane lipid fatty acid saturation decrease membrane fluidity in model and biologic membranes (42–44). However, the changes in saturation indices in rat microvillus membrane during development did not appear to counterbalance the rising cholesterol/phospholipid molar ratio which led to decreases in fluidity with increasing age in jejunum when examined at both temperatures or ileum when examined at 25°C. Additional membrane compositional changes with age, including alterations in the protein-to-lipid ratio, may have affected fluidity but these measurements were not performed as a portion of the current studies.

The explanation for the absence of a fluidity change in ileal microvillus membrane at 37°C in spite of changes in the cholesterol/phospholipid ratio is unclear. At 37°C, depolarizing rotations of diphenylhexatriene may be less hindered than at 25°C (45, 46). This hindrance may derive from changes that are occurring in the membrane associated with phase transition from a liquid crystalline phase to a crystalline gel around the thermotropic transition of 23–26°C (36, 47). During this transition, lipid cluster formation appears to develop with some lipids remaining in a fluid state while others are in a more solid phase (48). In addition, the lateral compressibility of the membrane increases (49). In this circumstance, the effect of one lipid species, i.e., cholesterol, may become predominant in its effect on fluidity and thereby lead to the alterations observed at 25°C which contrast with those at 37°C.

Microvillus membrane cholesterol and phospholipid content and fluidity in adult rats measured in the present study were comparable to findings from previous studies (47, 50–53). Postnatal changes in microvillus membrane total lipid, cholesterol, phospholipid, fatty acid species, and fluidity have previously been examined in the rabbit (36, 53). In the rabbit, the cholesterol/phospholipid molar ratio increased and fluidity declined with age as was observed in our studies in the rat. However, whereas cholesterol content was unchanged and phospholipid content declined in the rabbit, phospholipid content was unchanged and cholesterol rose in the rat with increasing age. The precise reason for these discrepancies is unknown but may be related to species differences; however, in both species, microvillus membranes became less fluid with increasing age.

Many enzyme and transport systems in mature species appear dependent upon membrane lipid composition and fluidity for expression. Our studies suggest that the expression of the ileal active bile salt transport "system" and its putative receptor may be expected to be altered by physico-chemical changes of the bulk phase of the microvillus membrane. This notion may be further corroborated by studies of ethanol-treated adult rats in which increasing membrane fluidity was associated with reduced ileal active taurocholate transport in microvillus membrane vesicles (54). However, our studies only allow the demonstration of an association rather than a cause-and-effect relationship between the development of ileal active bile salt transport and alterations in membrane fluidity. Caution in interpretation of our results must be exercised since the postnatal development of sucrase (an extrinsic enzyme) and coincident changes in jejunal microvillus membrane fluidity might be interpreted as being causally linked (55). In addition, changes in ileal microvillus membrane fluidity at 25°C but not 37°C

might raise questions regarding the physiologic significance of our findings. Future studies examining the effect of hormones and dietary influences in precocious or delayed postnatal development of ileal active bile salt transport and coincident changes in membrane fluidity may help clarify these relationships. ⬛

## REFERENCES

1. Hofmann, A. F. 1976. The enterohepatic circulation of bile acids in man. *Adv. Intern. Med.* **21:** 501–534.
2. Small, D. M., R. H. Dowling, and R. N. Redinger. 1972. The enterohepatic circulation of bile salts. *Arch. Intern. Med.* **130:** 552–573.
3. Lack, L., and I. M. Weiner. 1961. In vitro absorption of bile salts by small intestine of rats and guinea pigs. *Am. J. Physiol.* **200:** 313–317.
4. Schiff, E. R., N. C. Small, and J. M. Dietschy. 1972. Characterization of the kinetics of the passive and active transport mechanisms for bile acid absorption in the small intestine and colon of the rat. *J. Clin. Invest.* **51:** 1351–1362.
5. Krag, E., and S. F. Phillips. 1974. Active and passive bile acid absorption in man. Perfusion studies of the ileum and jejunum. *J. Clin. Invest.* **53:** 1686–1694.
6. Watkins, J. B., D. Ingall, P. A. Szczepanik, P. D. Klein, and R. Lester. 1973. Bile salt metabolism in the newborn: measurement of pool size and synthesis by stable isotope technique. *N. Engl. J. Med.* **288:** 431–434.
7. Watkins, J. B., P. Szczepanik, J. B. Gould, P. Klein, and R. Lester. 1975. Bile salt metabolism in the human premature infant. *Gastroenterology.* **69:** 706–713.
8. Watkins, J. B., A. L. Jarvenpaa, P. Szczepanik-Van Leeuwen, P. D. Klein, D. K. Rassin, G. Gaull, and N. C. R. Raiha. 1983. Feeding the low birth weight infant. V. Effects of taurine, cholesterol and human milk on bile acid kinetics. *Gastroenterology.* **85:** 793–800.
9. Heubi, J. E., W. F. Balistreri, and F. J. Suchy. 1982. Bile salt metabolism in the first year of life. *J. Lab. Clin. Med.* **100:** 127–136.
10. Heubi, J. E., and W. F. Balistreri. 1980. Bile salt metabolism in infants and children after protracted infantile diarrhea. *Pediatr. Res.* **14:** 943–946.
11. DeBelle, T. C., V. Vaupstras, B. B. Vitullo, L. R. Haber, E. Shaffer, G. G. Mackie, H. Owen, J. M. Little, and R. Lester. 1979. Intestinal absorption of bile salts: immature development in the neonate. *J. Pediatr.* **94:** 472–476.
12. Little, J. M., and R. Lester. 1980. Ontogenesis of intestinal bile salt absorption in the neonatal rat. *Am. J. Physiol.* **239:** G319–G323.
13. Lester, R., R. A. Smallwood, J. M. Little, A. S. Brown, G. J. Piasecki, and B. T. Jackson. 1977. Fetal bile salt metabolism. The intestinal absorption of bile salt. *J. Clin. Invest.* **59:** 1009–1016.
14. Heubi, J. E., and J. D. Fondacaro, 1982. Postnatal development of intestinal bile salt transport in the guinea pig. *Am. J. Physiol.* **243.** G189–G194.
15. Sandermann, H., Jr. 1978. Regulation of membrane enzymes by lipids. *Biochim. Biophys. Acta.* **515:** 209–237.
16. Shinitzky, M., H. Borochov, and W. Wilbrandt. 1980. Lipid fluidity as a physiological regulator of membrane transport and enzyme activities. *In* Membrane Transport in Erythrocytes. U. V. Lassen, H. H. Ussing, and J. D. Wieth, editors. Alfred Benson Symposium 14. Munksgaard, Copenhagen. 91–107.
17. Shinitzky, M., and Y. Barenholz. 1978. Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim. Biophys. Acta.* **515:** 367–394.
18. Kapitulnik, J., M. Tshevshedsky, and Y. Barenholz. 1979. Fluidity of the rat liver microsomal membrane: increase at birth. *Science.* **206:** 843–844.
19. Kutchai, H., Y. Barenholz, T. E. Ross, and D. E. Wermer. 1976. Developmental changes in plasma membrane fluidity in chick embryo heart. *Biochim. Biophys. Acta.* **436:** 101–112.
20. Gudmundsdottir, A., and S. Gudbjarnason. 1983. Neonatal changes in fatty acid profile of phospholipids in rat heart muscle. *Biochim. Biophys. Acta.* **752:** 284–290.
21. Schmitz, J., H. Preiser, D. Maestracci, B. K. Ghosh, J. J. Cerda, and R. K. Crane. 1973. Purification of human intestinal brush border membrane. *Biochim. Biophys. Acta.* **323:** 98–112.
22. Kessler, M., O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Semenza. 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. *Biochim. Biophys. Acta.* **506:** 136–154.
23. Folch, J., M. Lees, G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
24. Barenholz, Y., E. J. Patzer, N. F. Moore, and R. R. Wagner. 1977. Cholesterol oxidase as a probe for studying membrane composition and organization. Adv. Exp. Biol. Med. **101:** 45–56.
25. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 469–471.
26. MacGee, J., and K. G. Allen. 1974. Preparation of methyl esters from the saponifiable fatty acids in small biological specimens for gas–liquid chromatographic analysis. *J. Chromatogr.* **100:** 45–42.
27. Shinitzky, M., and Y. Barenholz. 1974. Dynamics of the hydrocarbon layer in liposomes of lecithin and sphingomyelin containing dicetylphosphate. *J. Biol. Chem.* **249:** 2652–2657.
28. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurements with Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
29. Dahlqvist, A. 1964. Method for assay of intestinal disaccharidases. *Anal. Biochem.* **7:** 18–25.
30. Pekarthy, J. M., J. Short, A. I. Lansing, and I. Lieberman. 1972. Function and control of liver alkaline phosphatase. *J. Biol. Chem.* **247:** 1767–1774.
31. Keefe, E. B., B. F. Scharschmidt, N. M. Blankenship, and R. K. Ockner. 1979. Studies of relationships among bile flow, liver, plasma membrane Na,K-ATPase and membrane microviscosity in the rat. *J. Clin. Invest.* **64:** 1590–1598.
32. Cleland, W. W. 1979. The statistical analysis of enzyme

kinetic data. *Methods Enzymol.* **63:** 103–138.

33. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th Ed. Iowa State University Press, Ames, IA.

34. Koldovsky, O. 1981. Developmental, dietary and hormonal control of intestinal disaccharidases in mammals (including man). *In* Carbohydrate Metabolism and its Disorders. Vol. 3, P. J. Randle et al., editors. Academic Press, New York. 482–513.

35. Henning, S. J. 1981. Postnatal development: coordination of feeding, digestion and metabolism. *Am. J. Physiol.* **241:** G199–G214.

36. Schwarz, S. M., S. Ling, B. Hostetler, J. P. Draper, and J. B. Watkins. 1984. Lipid composition and membrane fluidity in the small intestine of the developing rabbit. *Gastroenterology.* **86:** 1544–1551.

37. Di Costanzo, G., G. Duportail, A. Florentz, and C. Leray. 1983. The brush border membrane of trout intestine: influence of its lipid composition on ion permeability, enzyme activity and membrane fluidity. *Molec. Physiol.* **4:** 279–290.

38. Neelands, P. J., and M. T. Clandinin. 1983. Diet fat influences liver plasma-membrane lipid composition and glucagon-stimulated adenylate cyclase activity. *Biochem. J.* **212:** 573–583.

39. Shattil, S. J., and R. A. Cooper. 1976. Membrane microviscosity and human platelet function. *Biochemistry.* **15:** 4832–4837.

40. Cooper, R. A., J. R. Durocher, and M. H. Leslie. 1977. Decreased fluidity of red cell membrane lipids in abetalipoproteinemia. *J. Clin. Invest.* **60:** 115–121.

41. Shinitzky, M., and M. Inbar. 1976. Microviscosity parameters and protein mobility in biological membranes. *Biochim. Biophys. Acta.* **433:** 133–149.

42. Wilson, G., S. P. Rose, and C. F. Fox. 1970. The effect of membrane lipid unsaturation on glycoside transport. *Biochem. Biophys. Res. Commun.* **38:** 617–623.

43. McVey, E., J. Yguerabide, D. C. Hanson, and W. R. Clark. 1981. The relationship between plasma membrane lipid composition and physical-chemical properties. Biochim. Biophys. Acta. **642:** 106–118.

44. Holloway, C. T., and P. W. Holloway. 1977. The dietary regulation of stearyl coenzyme A desaturase activity and membrane fluidity in the rat aorta. *Lipids.* **12:** 1025–1031.

45. Chen, L., R. E. Dale, S. Roth, and L. Brand. 1977. Nanosecond time-dependent fluorescence depolarization of diphenylhexatriene in dimyristoyllecithin vesicles and determination of "microviscosity". *J. Biol. Chem.* **252:** 2163–2169.

46. Lakowicz, J. R., and F. G. Prendergast. 1978. Quantitation of hindered rotations of diphenylhexatriene in lipid bilayers by differential polarized phase fluorometry. *Science.* **200:** 1399–1401.

47. Schachter, D., and M. Shinitzky. 1977. Fluorescence polarization studies of rat intestinal microvillus membranes. *J. Clin. Invest.* **59:** 536–548.

48. Lee, A. G. 1975. Functional properties of biological membranes: a physical-chemical approach. *Prog. Biophys. Molec. Biol.* **29:** 3–56.

49. Linden, C. D., K. L. Wright, H. M. McConnell, and C. F. Fox. 1973. Lateral phase separations in membrane lipids and the mechanism of sugar transport in *Escherichia coli. Proc. Natl. Acad. Sci.* **70:** 2271–2275.

50. Christiansen, K., and J. Carlsen. 1981. Microvillus membrane vesicles from small intestine. Purity and lipid composition. *Biochim. Biophys. Acta.* **647:** 188–195.

51. Hauser, H., K. Howell, R. M. C. Dawson, and D. E. Bowyer. 1980. Rabbit small intestinal brush border membrane. Preparation and lipid composition. *Biochim. Biophys. Acta.* **602:** 567–577.

52. Forstner, G. G., K. Tanaka, and K. J. Isselbacher. 1968. Lipid composition of the isolated rat intestinal microvillus membrane. *Biochem. J.* **109:** 51–59.

53. Pang, K. Y., J. L. Bresson, and W. A. Walker. 1983. Development of the gastrointestinal mucosal barrier. Evidence for structural differences in microvillus membranes from newborn and adult rabbits. *Biochim. Biophys. Acta.* **727:** 201–208.

54. Hunter, C. K., L. L. Treanor, J. P. Gray, S. A. Halter, A. Hoyumpa, and F. A. Wilson. 1983. Effects of ethanol in vitro on rat intestinal brush-border membranes. *Biochim. Biophys. Acta.* **732:** 256–265.

55. Brasitus, T. A., D. Schachter, and T. G. Mamouneas. 1979. Cholesterol biosynthesis and modulation of membrane cholesterol and lipid dynamics in rat microvillus membranes. *Biochemistry.* **18:** 4136–4144.