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The Role of Glucocorticoids in the Postnatal Development of Ileal Active Bile Salt Transport

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ABSTRACT. The role of glucocorticoids in the regulation of the postnatal development of ileal active bile salt transport was examined in the rat using the villus technique. Ileal taurocholate uptake was initially by passive transport alone on day 14 and 16 which changed to an active and passive transport mechanism at 18 days which persisted thereafter. The Michaelis-Menten constant (Km, mM) was unchanged between days 18 (0.67 ± 0.12 mM), 20 (0.84 ± 0.25 mM), 21 (0.49 ± 0.05 mM), 28 (0.59 ± 0.06 mM), and 49 (0.50 ± 0.05 mM) whereas the apparent maximal velocity nmol/mg(dry wt)/min declined after a peak at 18 days (18.17 ± 1.92 on day 18, 16.14 ± 1.89 on day 20, 14.65 ± 0.52 on day 21, 11.40 ± 0.35 on day 28, and 10.51 ± 0.32 on day 49). Adrenalectomy performed in sucklings on day 14 with taurocholate transport studies on day 21 and in adults on day 42 studied on day 49 resulted in reductions in uptake at most study concentrations but no change in the Km (1.33 ± 0.54 in sucklings and 0.75 ± 0.14 mM in adults) or apparent maximal velocity [11.78 ± 2.06 in sucklings and 9.24 ± 0.65 nmol/mg (dry wt)/min in adults]. Treatment of sucklings with corticosterone (5 mg/100 g body weight) on days 10–13 with study on day 14 and 16 did not produce precocious development of ileal active taurocholate transport; however, corticosterone treatment led to apparent increases in ileal permeability to taurocholate in both sucklings and adults. Glucocorticoids appear to play a minor, if any, role in the physiologic postnatal development of ileal active bile salt transport. (*Pediatr Res* 19: 1147–1151, 1985)

Abbreviations

Vapp, apparent maximal velocity
Km, Michaelis-Menten constant
Papp, apparent monomeric permeability

The postnatal development of ileal active bile salt transport parallels the rise in jejunal sucrase activity in the rat. During the 3rd postnatal wk, ileal bile salt transport changes from a solely passive mechanism to an active Na-dependent cotransport system (1). At the same time sucrase activity rises markedly from very low concentrations (2, 3).

The factors that mediate the ontogeny of intestinal enzyme and transport systems are incompletely understood at present. Various factors such as diet, hormones such as glucocorticoids and thyroxine, and genetic preprogramming have been extensively explored as modulators of the postnatal development of intestinal disaccharidases (4–7). However, factors involved in the development of intestinal transport systems remain largely unexplored. The present study was designed to evaluate the role of glucocorticoids in the development of ileal active bile salt transport in the developing rat.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) or Harlan Industries (Indianapolis, IN). Suckling animals were obtained from the supplier with their respective dam 3–4 days before initiation of injections or surgery to allow adaptation to our animal facility prior to the study. Similarly, 5-wk-old rats were allowed a period of adaptation prior to injections or surgery at age 6 wk. Animals were maintained in our animal facility with diurnal light cycling and allowed to suckle or eat Purina Rat Chow (St. Louis, MO) and water *ad libitum* as dictated by age 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 (8). Segments of ileum and jejunum were incubated at 37° C for 2 min in individual gassed 50-ml incubation flasks containing 5.0 ml Krebs buffer and 10 mM glucose containing 0.1, 0.25, 0.5, 1.0, 2.0, or 3.5 mM sodium taurocholate (Calbiochem, La Jolla, CA) and tracer 24-^{14}C-taurocholic acid (New England Nuclear, Boston, MA; Sp Act 52.0 mCi/mmol). After a 2-min incubation, the tissue was rinsed, frozen, and lyophilized overnight. The villi were chipped free of the underlying crypt, submucosal, and muscular layers. The villi were solubilized with Soluene-100 (Packard Instruments, Downer's Grove, IL), scintillation cocktail added (Aquassure, New England Nuclear), and radioactivity counted in a Beckman LS 7500 scintillation spectrometer (Fullerton, CA). Previous studies in guinea pig and rat had validated that uptake was linear with time up to 3.5–4.0 min and that comparable relationships between tissue dry weight and protein concentration were present at various ages tested.

OTHER MEASUREMENTS

The small bowel from the ligament of Trietz to the cecum was removed and divided into four equal segments. The second segment, designated midjejunum, was flushed with ice-cold 0.9% NaCl and homogenized in 0.15 M NaCl using a Ten-Broeck hand homogenizer. Midjejunum was selected because of superior consistency of results of sucrase measurements in this segment compared with other portions of small bowel (Koldovsky O, personal communication). Protein was measured by the method

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of Lowry *et al.* (9) using bovine serum albumin (Sigma Chemical Company, St. Louis, MO) as a standard. Sucrase was measured by the method of Dahlqvist using the coupled enzyme reaction of peroxidase-glucose oxidase (Sigma Chemical Company) (10).

Serum corticosterone was measured by radioimmunoassay (Radioassay Systems Laboratories, Carson, CA).

STUDY DESIGN

Normal Development. Sprague-Dawley pups suckled until spontaneous weaning and were killed at age 14 ($n = 18$), 16 ($n = 11$), 18 ($n = 9$), 20 ($n = 13$), and 21 ($n = 15$) days.

Adrenalectomy. Suckling rats. Litters were culled to 10 pups on day 10. Five pups were adrenalectomized, after anesthesia with diethyl ether, through bilateral dorsal incisions on day 14. The remaining littermates were anesthetized, had bilateral dorsal skin and muscle incisions, and served as sham controls. All pups suckled on dams given 0.9% NaCl in the drinking water until sacrifice on day 21. Overall mortality for adrenalectomized pups was approximately 10%. The weight of adrenalectomized and shams was comparable at time of operation; however, adrenalectomized rats gained only 9.9 g *versus* shams who gained 18.8 g ($p < 0.001$).

Adults. Adrenalectomy was performed, under diethyl ether anesthesia, through bilateral dorsal incisions on day 42. Rats received chow and 0.9% NaCl in drinking water until sacrifice on day 49. Overall mortality for adults was 10%.

Corticosterone Treatment. Suckling rats. Litters were culled to 10 pups on day 10. One half of the pups received intraperitoneal injection of corticosterone (Sigma Chemical Company) in a dose 5 mg/100 g body weight, dissolved in sesame oil, on days 10, 11, 12, and 13 with sacrifice on day 14 and 16. The remaining half of the litters received intraperitoneal injections of the vehicle (sesame oil) on days 10, 11, 12, and 13 with sacrifice on day 14 or 16. Initial weights of sham and treated animals were similar (19.6 *versus* 19.7 g); however, corticosterone treated animals were significantly smaller ($p < 0.001$) at 14 days (24.7 ± 0.4 g) compared to shams (26.2 ± 0.5 g). Overall mortality in all corticosterone and vehicle treated pups was less than 4%.

Adults. Corticosterone (5 mg/100 g body weight) was given on days 45, 46, 47, and 48 to rats ($n = 10$) with sacrifice on day 49. There were no deaths in this group.

GENERAL CONSIDERATIONS

The design of the corticosterone treatment and adrenalectomy studies was based upon studies on the postnatal development of sucrase (3). Both the postnatal development of sucrase and ileal active taurocholate transport occur during the 3rd postnatal wk. Studies giving corticosterone on days 10, 11, 12, and 13 with study on day 14 or 16 and adrenalectomy on day 14 with study on day 21 might be anticipated to affect ileal active transport as they had previously been shown to affect sucrase (4, 6, 7, 11). Relatively high doses of corticosterone (5 mg/100 g body weight) were given to insure an adequate pharmacologic effect. Parallel studies in adults were performed to determine whether the observed effects of corticoids were present only during development or were observed at all ages.

STATISTICAL CALCULATIONS

All results are expressed as mean ± SE. Bile salt uptake (J) was calculated using the equation $J(\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg dry wt}^{-1}) = \text{cpm/sp act time (min)} \times \text{dry wt (mg)}$ (15). Vapp ($\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg dry weight}^{-1}$) and the apparent Km (mM) were calculated using a computer program available through Clinfo (Division of Research Resources, National Institutes of Health, Bethesda, MD) that permitted a weighted least-squares fit of individual data points (12). Passive ileal and jejunal permeability (Papp) of monomeric concentrations of taurocholate were calculated using the equation Papp ($\text{nmol}\cdot\text{min}^{-1}$ mg dry $\text{wt}^{-1}\cdot\text{mM}^{-1}$) equals J/C where C equals the monomer taurocholate concentration. Differences between apparent Km and Vapp in different experimental conditions were tested for significance using the Z test. All other comparisons were made using a two tailed Student's t test (13).

RESULTS

Taurocholate Transport. Normal development. Our previous studies had demonstrated that ileal active taurocholate transport was first evident in the rat on postnatal day 21. In the present studies, we examined the time sequence of development during the 3rd postnatal wk. On days 14 and 16, the relationship between taurocholate uptake in both ileum and jejunum and the incubation medium concentration was linear suggesting that only passive transport mechanisms were present (Fig. 1). On day 14, uptake rates for both ileum and jejunum were comparable ($p =$ *NS*) at all incubation medium concentrations. However, on day 16 uptake rates differed between the ileum and jejunum at all concentrations examined (0.1, 0.25, 0.5, 2.0; all $p < 0.01$; and 3.5; $p < 0.05$) except 1.0 mM ($p =$ NS). After 18 days, ileal uptake assumed the appearance of a rectangular hyperbola with respect to incubation medium concentration while jejunal uptake remained linear. Calculated transport characteristics (Km and Vapp) were derived. There were no significant differences between the Km at 18 days (0.67 ± 0.12 mM), 20 days (0.84 ± 0.25 mM), 21 days (0.49 ± 0.05 mM) and from previous studies, day 28 (0.59 ± 0.06 mM) and day 49 (0.50 ± 0.05 mM) (13).

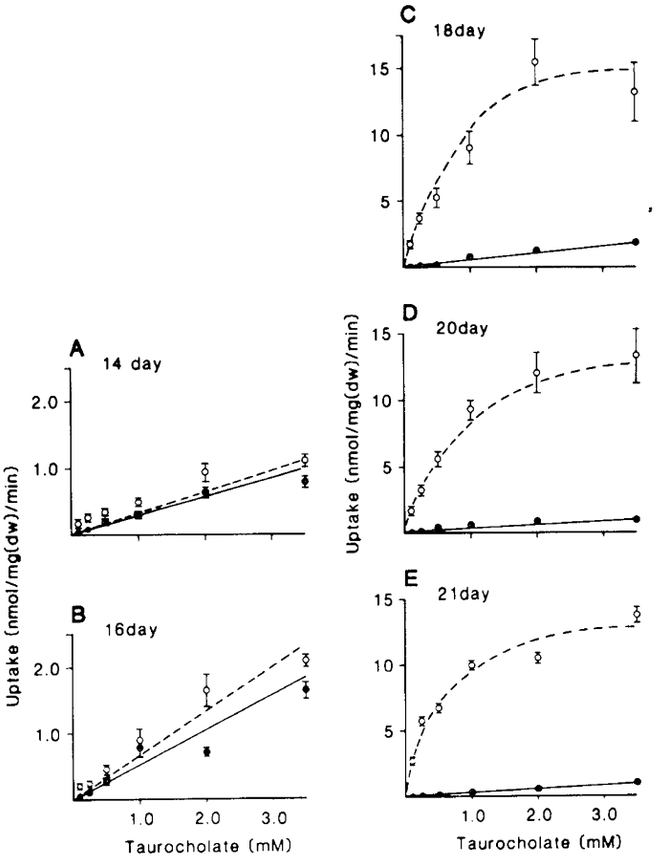

Fig. 1. Ileal --o-- and jejunal —♦— uptake of taurocholate at various ages. Note similar ileal and jejunal uptake rates at 14 days with divergence of rates at 16 days. A curvilinear relationship between ileal uptake and concentration is present at 18, 20, and 21 days indicating the presence of active transport while jejunal uptake remains linear with respect to concentrations. ♦ = mean ± SE.

Table 1. *Characteristics of adrenalectomized and corticosterone-treated rats*

Group	*n*	Body wt (g)	Serum corticosterone (ng/ml)	Km (mM)	Vapp [nmol/ mg(dw)/min]
Control					
14 day	18	26.2 ± 0.5	44.0 ± 4.0		
21 day	15	47.0 ± 1.2	311.5 ± 19.5	0.49 ± 0.05	14.65 ± 0.52
49 day	18	184.3 ± 7.1	328.5 ± 24.6	0.50 ± 0.05	10.51 ± 0.32
Adrenalectomized					
21 day	19	39.2 ± 1.2*	24.4 ± 3.7*	1.33 ± 0.54	11.78 ± 2.06
49 day	10	150.9 ± 5.6*	14.7 ± 0.9*	0.75 ± 0.14	9.24 ± 0.65
Corticosterone treated					
14 day	30	24.7 ± 0.4†	ND‡		

* $p < 0.001$ *vs.* age-matched control.
† $p < 0.05$ *vs.* age-matched control.
‡ Not done.

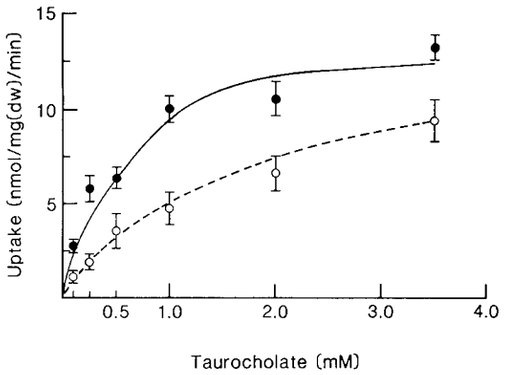

Fig. 2. Ileal taurocholate uptake in 3-wk-old adrenalectomized rats --o-- compared to 3-wk-old shams —♦—. ♦ = mean ± SE. Please note difference in *vertical axis* compared to Figure 1.

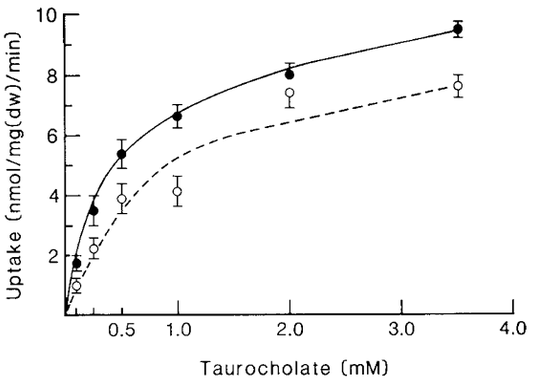

Fig. 3. Ileal taurocholate uptake in 7-wk-old adrenalectomized rats --o-- compared to 7-wk-old shams —♦—. ♦ = mean ± SE.

Similarly, although initially higher the Vapp did not differ significantly between 18 day [18.17 ± 1.92 nmol/mg (dry weight/ min], 20 day [16.14 ± 1.89 nmol/mg (dry weight)/min], and 21 day [14.65 ± 0.52 nmol/mg (dry weight)/min]. However, the Vapp at 18 days was significantly higher than that at age 28 days [11.40 ± 0.35 nmol/mg (dry weight)/min, $p < 0.001$] and than that in adults [10.51 ± 0.32 nmol/mg (dry weight)/min, $p < 0.001$] (1).

Adrenalectomy. All rats, both suckling and adult, were effectively adrenalectomized as determined by two independent criteria. No residual adrenal tissue was present at the time of sacrifice and serum corticosterone concentrations were markedly reduced compared to controls (Table 1). The concentrations found in both 21 day and 49 day adrenalectomized rats were both below the concentration found in 2-wk-old pups, a time which precedes the normal postnatal rise in serum corticosterone levels to concentrations found in the adult (14). Adrenalectomized suckling and adult rats were significantly smaller ($p < 0.001$) than controls (Table 1). In the suckling rats, a curvilinear relationship between ileal taurocholate uptake and concentration was found, suggesting the presence of ileal active transport while jejunal uptake remained linear. The Km and Vapp were not significantly different from control 21 day old rats (Table 1); however, individual ileal uptake rates were significantly lower than controls at all incubation concentrations (0.10, 0.25, 1.0, and 2.0 mM; all $p < 0.001$; and 0.5 and 3.5, both $p = 0.02$) (Fig. 2). Similarly, despite comparable ileal Km and Vapp values for adrenalectomized and control adults, there were consistent significant (all $p < 0.01$ except 2.0 mM) reductions in ileal uptake rates at all study concentrations (Table 1, Fig. 3). There was a significant effect of adrenalectomy on the development of sucrase activity. Sucrase activity measured in midjejunal homogenates from 21-day-old adrenalectomized rats was significantly lower ($p < 0.001$) than similarly aged shams (16.5 ± 1.7 *versus* 94.5 ± 8.3 μmol/g protein/min, respectively). Adrenalectomy had no significant effect on jejunal sucrase activity in adult rats with sucrase activity of 56.9 ± 4.1 μmol/g protein/min in controls and 70.7 ± 8.5 μmol/g protein/min in their adrenalectomized counterparts.

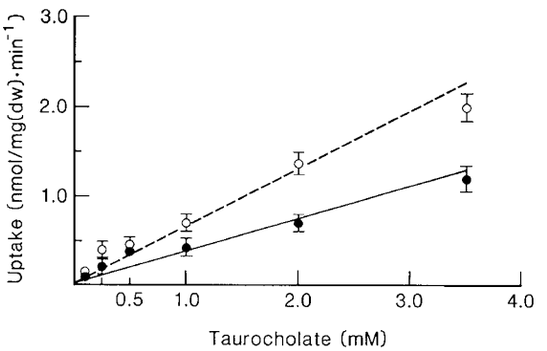

Fig. 4. Comparison between ileal --o-- and jejunal —♦— uptake of taurocholate in 14-day-old rats treated with corticosterone (5 mg/100 g body weight) on days 10, 11, 12, and 13.

Corticosterone treatment. Corticosterone-treated rats who were sacrificed on day 14 were significantly smaller ($p < 0.05$) than vehicle-treated shams (Table 1). Corticosterone treatment resulted in a significant increase in ileal taurocholate uptake rates at all concentrations (all $p < 0.01$) except 0.1 mM (Fig. 4). Although uptake was increased, the relationship between uptake and medium concentration remained linear with corticosterone therapy suggesting only passive transport for taurocholate was present. The findings also suggest that ileum from corticosterone treated rats became more permeable to taurocholate. The Papp was significantly increased ($p < 0.0001$) in ileum of corticosterone treated pups compared to controls (Table 2). Significant difference in uptake were only noted between corticosterone-treated and vehicle-treated jejunum at medium concentrations of 2.0 and 3.5 mM ($p < 0.001$); however, significant changes ($p < 0.001$) in Papp were observed compared to controls. To determine if corticosterone was capable of inducing ileal active transport if additional time after treatment were allowed, we studied corticosterone treated animals at age 16 days. As depicted in Figure 5, ileal uptake rates on day 16 were significantly higher than found in age-matched controls and higher than treated animals on day 14 at all concentrations ($p < 0.05$ or less) except 0.25 mM. However, uptake rates remained linear with respect to taurocholate concentrations. Fourteen- and 16-day controls had very low jejunal sucrase activities (5.9 ± 0.6 and 7.0 ± 1.6 μmol/g protein/min, respectively). With corticosterone therapy, sucrase activity rose significantly (both $p < 0.001$) in 14 (30.0 ± 4.4 μmol/g protein/min) and 16 (75.5 ± 11.2 μmol/g protein/min) day olds. Corticosterone treatment in adult rats led to similar increases in ileal uptake. The curvilinear relationship between uptake and medium concentration was preserved; however, marked increases in uptake rates at higher concentration were observed (data not shown). Corticosterone treatment in adults had no significant effect on jejunal sucrase activity. Seven-week-old shams had jejunal sucrase activity of 56.9 ± 4.1 μmol/g protein/min compared to 63.5 ± 8.6 μmol/g protein/min. in similar aged corticosterone-treated rats.

Table 2. *Apparent jejunal and ileal permeability coefficients in sham and corticosterone-treated sucklings*

			Papp ($nmol \cdot min^{-1} \cdot mg^{-1} \cdot mM^{-1}$)	
Age	*n*	Treatment	Jejunum	Ileum
14 day	26	Sham	0.273 ± 0.010	0.274 ± 0.011
14 day	30	Corticosterone	0.459 ± 0.016*	0.999 ± 0.057*,†

* $p < 0.0001$ *vs.* sham.
† $p < 0.0001$ *vs.* jejunum.

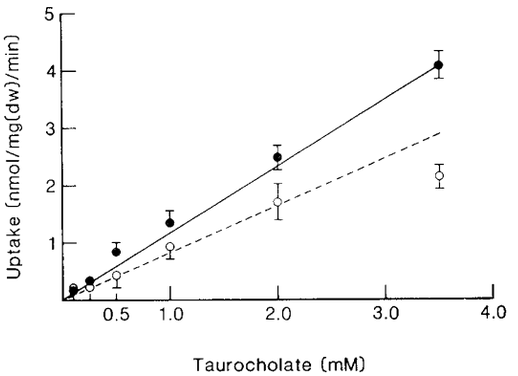

Fig. 5. Ileal taurocholate uptake in 16-day-old —●— and 14-day-old --○-- rats treated with corticosterone (5 mg/100 g body weight) on days 10, 11, 12, and 13.

DISCUSSION

In the rat, the postnatal development of ileal active taurocholate transport occurs during the 3rd postnatal wk (1, 15). In the present study we have demonstrated, using the villus uptake method, that ileal uptake changed from solely passive means on the 14th and 16th postnatal days to an active and passive process on day 18 which persisted thereafter. It appears as if the binding affinity for taurocholate is unchanged with age after the development of ileal active transport as evidenced by similar Km at age 18, 20, 21, 28, nd 42–56 days. The number of ileal taurocholate binding sites may decrease with age as suggested by the decrease in Vapp which declined from a peak at age 18 days.

The factors which modulate the postnatal development of intestinal enzyme and transport systems are incompletely understood. Dietary and hormonal factors and genetic preprogramming have all been explored to determine their role(s) in the development of intestinal disaccharidases (4–7). The role of glucocorticoids in the development of sucrase has been extensively examined. Serum concentrations of the principal glucocorticoid of the rat, corticosterone, rise in parallel with the postnatal development of sucrase activity (14). In sucklings, corticoid treatment leads to a precocious rise in sucrase activity while adrenalectomy blunts the normal postnatal rise (2, 11). However, recent studies suggest that glucocorticoids are not necessary for the postnatal development of sucrase (16).

The results of the present study suggest that the postnatal development of ileal active taurocholate transport occurs independently of glucocorticoids. This conclusion is supported by several experimental findings: 1) Adrenalectomy in both suckling and adult rats did not eliminate ileal active bile salt transport. Although individual uptake rates at various incubation concentrations were reduced, the kinetic characteristics (Km, Vapp) were unaffected at both ages. In this regard, it appears as if adrenalectomy affects ileal active transport at all ages rather than simply affecting its development. Since adrenalectomy in both suckling and adult rats led to growth retardation, it is possible that the observed effects are nutrition related rather than glucocorticoid related (17, 18). Reductions in bile salt pools following adrenalectomy may also indirectly lead to alterations in ileal active taurocholate transport (19). In contrast, sucrase activity was markedly reduced with adrenalectomy in sucklings but unaffected in adults. The observed changes in sucrase activity with adrenalectomy corroborate previous observations (11). In addition, the biologic effects of adrenalectomy (alterations in sucrase activity) coupled with marked serum corticosterone redutions confirm the adequacy and validity of the adrenalectomy model in these studies. 2) Treatment with pharmacologic doses of corticosterone (5 mg/100 g body weight) for 4 days to suckling rats failed to cause precocious appearance of ileal active bile salt transport. Instead, when studied after corticosterone treatment at both age 14 and 16 days, taurocholate was transported by passive means only; however, significant increases in uptake rates related to increased passive permeability to taurocholate were observed at both 14 and 16 days compared to untreated controls of comparable age. Corticosterone treatment of adults did not affect ileal active transport but did increase passive (jejunal) uptake and the presumed passive component of ileal uptake. In corticosterone-treated sucklings (14 and 16 days), sucrase activity was markedly increased compared to controls and treatment of adults had no effect on sucrase activity. These observations corroborated the known biologic effects of glucocorticoid treatment on sucrase validating it as a model of corticoid excess.

Glucocorticoid treatment has been shown to promote precocious development of a limited number of intestinal transport systems. Magnesium, calcium, and glucose-depenent Na transport and vitamin B_{12} absorption are affected by glucocorticoid treatment of suckling species (20–22). Little and Lester (15) have suggested that ileal active transport developed precociously in

suckling rats treated with dexamethasone. The reasons for the discrepancies between our findings and those of Little and Lester (15) may relate to methodologic differences. Confusion regarding duration of incubation, concentration of taurocholate in incubation medium, and expression of results as ratios of tissue concentration to incubation medium make interpretation of the results of Little and Lester (15) difficult. However, the most significant difference may have been the timing of and type of glucocorticoid administered. Using the villus uptake method, corticosterone treatment on days 10–13 in pharmacologic doses large enough to produce precocious development of sucrase did not stimulate premature development of ileal active transport while Little and Lester (15) treated dams or sucklings for 6–9 days after birth. The timing of our studies was based on the previous observations of normal development of ileal active transport on day 18 and the normal postnatal rise in serum corticosterone in the rat (16). Corticosterone was used because it is in the principal corticoid in the rat although its glucocorticoid potency is recognized to be low. Although the use of a more potent glucocorticoid such as dexamethasone may have led to different results, corticosterone treatment did produce a significant pharmacologic effect on the development of sucrase and therefore should have been equally effective in altering the postnatal development of ileal active bile salt transport. Although treatment of sucklings with pharmacologic doses of corticosterone on days 10, 11, 12, and 13 may be appropriate for evaluation of its role in normal physiology, treatment with more potent glucocorticoids at the same or other times may lead to different effects on the development of ileal active taurocholate transport. Since we were unable to stimulate premature appearance on day 14 or 16 using our technique and the principal glucocorticoid of the rat, it would appear as if corticoids play a minimal role in the normal development of ileal active taurocholate transport under physiologic conditions in the rat.

Both term and preterm human infants have significant steatorrhea during the first postnatal months when compared to older children and adults. Reduced intraluminal bile salt concentrations and pancreatic lipase in newborns may contribute significantly to this reduced fat absorption. The observed bile salt insufficiency can be explained by 1) reduced pool sizes, 2) reduced hepatic synthesis and immature feedback inhibition mechanisms, and 3) inefficient enterohepatic cycling (impaired hepatic and intestinal transport mechanisms) (23). Preterm infants whose mothers received dexamethasone or phenobarbital were found to have larger bile salt pools, increased bile salt synthesis, and reduced turnover rates for cholate and chenodeoxycholate compared to untreated controls (24). However, additional studies by the same investigators failed to confirm these observations (25). Dexamethasone administered to rat dams during gestation increases taurocholate pools in their offspring presumably mediated by increased hepatic synthesis and/or enhanced hepatic transport (26, 27). Little and Lester (15) demonstrated precocious increases in ileal bile salt transport in pups of mothers treated with dexamethasone or in pups who were treated immediately after birth. Our studies failed to show the appearance of ileal active transport when rats were treated from days 10–13. Although glucocorticoids affect taurocholate pool size, hepatic synthesis, and ileal transport when pups are treated during gestation or shortly after birth, no similar effect has been consistently observed in humans. If glucocorticoids were to prove useful in precociously stimulating maturation of hepatic and intestinal bile salt metabolism in the human, they most likely would have to be administered during gestation or in the first postnatal days. Since glucocorticoid administration beyond 10 days did not precociously stimulate the development of ileal active transport, we may assume that manipulations performed around this age which might stimulate corticosterone secretion (*e.g.* thyroidectomy, injections) would not confound the observed results.

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