

# Intestinal permeability in the newborn

L T WEAVER, M F LAKER, AND R NELSON

Departments of Child Health and Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne

**SUMMARY** Passive intestinal permeability in 33 newborn babies was studied using feeds containing lactulose and mannitol. Each marker is thought to pass across the gut wall by a different route; lactulose by a paracellular and mannitol by a transcellular pathway. Neither is metabolised and both are wholly and solely excreted by the kidney; urinary recovery is a measure of the intestinal uptake. Babies born before 34 weeks' gestation exhibited a higher intestinal permeability to lactulose than more mature babies, and all preterm babies showed an appreciable decline in lactulose absorption during the first week of oral feeds. Babies of 34 to 37 weeks' gestation achieved a 'mature' intestinal permeability to lactulose within four days of starting oral feeds. These findings may reflect the immaturity of the gut of the preterm baby rather than a process essential to adaptation to enteral nutrition.

One of the major challenges faced by the newborn baby is the abrupt transition from parenteral to enteral nutrition. By the third trimester of pregnancy the gut seems to be anatomically prepared,<sup>1</sup> but after oral feeding begins, considerable physiological and morphological changes take place.<sup>2</sup> These adaptations to extrauterine nutrition all contribute to the major function of the gut—absorption. Presented with food for the first time, however, the gut must distinguish the essential from the harmful, and balance selection with exclusion.

The gut of a newborn infant, especially a preterm infant, is permeable to intact sugars<sup>3</sup> and proteins.<sup>4</sup> These may be absorbed in considerable amounts for a period after birth, but in animals and man reduction in uptake seems to follow the onset of enteral nutrition.<sup>5</sup> We adopted and developed a method used by Beach *et al*<sup>6</sup> to study changes in intestinal permeability to lactulose and mannitol after the onset of oral feeding in neonates. These passively absorbed markers are thought to cross the intestinal wall by different routes, lactulose by paracellular and mannitol by transcellular pathways.

## Materials, patients, and methods

**Markers.** The markers lactulose and mannitol were chosen because they are both hydrophilic, lipophobic molecules already present in some infant milk formulas. Up to 6% of lactose may be converted to lactulose during terminal sterilisation used in the preparation of 'ready to feed' liquid infant milks.<sup>7</sup>

Our own analyses also showed measurable amounts of mannitol in some infant milks, up to approximately 2 mmol/l (37 mg/100 ml) in Wysoy granulated feed.

Neither marker is hydrolysed by intestinal brush border enzymes, nor has affinity for any active transport system.<sup>8</sup> The upper small intestine is the main site of marker absorption in adults.<sup>9</sup> We have studied the rate of urinary lactulose and mannitol excretion of a catheterised infant with normal gastrointestinal and renal function. During a study lasting 48 hours the ratio of lactulose to mannitol present in serial two hourly urine aliquots obtained while the baby received regular four to five hourly oral test feeds containing the markers in a concentration ratio of 7:1, became constant within two hours of the first test feed, and remained so until 6 hours after the last. Less than one per cent of ingested lactulose and five per cent of mannitol was absorbed from the test feeds.

Neither marker is metabolised within the body, though endogenous production of mannitol has been suggested in adults.<sup>10</sup> We found neither lactulose nor mannitol in the urine of babies fed formulas that were free of either marker. The compartmental distribution of both markers is effectively limited to the extracellular fluid space, though mannitol penetrates erythrocytes slowly *in vitro*.<sup>10</sup>

After intravenous injection both markers are wholly and solely excreted by the kidney with clearances close to that of creatinine.<sup>10</sup> Urinary lactulose recovery after intravenous injection was

found to approach 100% in adults and was greater than 92% over 24 hours in an anencephalic neonate.<sup>11</sup> Both markers are broken down by colonic flora to fatty acids, hydrogen, and carbon dioxide.<sup>12</sup>

**Patients and methods.** Thirty three newborn Caucasian babies were studied from birth to age 7 days. All were patients within a single maternity hospital in Newcastle and received standard medical care throughout the study, which was performed with the informed consent of parents and approval of the local ethical committee. Their gestational ages, which ranged from 27 to 41 weeks, were calculated from maternal menstrual history, ultrasound scan, and morphological characteristics at birth.<sup>13</sup>

The infants received regular two to four hourly oral feeds containing the markers in a concentration ratio of 5:1. All preterm and some term babies received SMA Gold Cap concentrated liquid feed (Wyeth Laboratories), the rest were given Milumil (Milupa). The lactulose and mannitol contents of these milks were measured by gas liquid chromatography and appropriate amounts of the markers were added to all feeds so that babies received milk containing 5.8 mmol/l (200 mg/100 ml) lactulose and 2.2 mmol/l (40 mg/100 ml) mannitol. The osmolality of feeds was unchanged. We have studied over 70 preterm and term babies using these test feeds without noting any adverse effects.

Those babies whose oral feeding was delayed because of prematurity or respiratory disease received 10% dextrose intravenously until oral feeding was begun. Feeds were given by orogastric tube or bottle, according to the baby's gestational age and condition, but no baby's first oral feed was delayed longer than four days after birth. No baby suffered severe birth asphyxia. Seven babies required assisted ventilation for respiratory distress syndrome. Five babies were excluded from the study; three had enterocolitis, one had congenital cytomegalovirus infection, and one died of septicaemia aged 3 days.

After 24 hours, when a steady state of rate of marker input and output had been reached, a random urine sample was collected daily for 7 days from preterm babies, and until discharge from term babies. Urine samples were stored at -20°C with 0.1 ml 10% sodium merthiolate for analysis of marker and creatinine concentrations.

Marker contents of the urine specimens were measured using a gas liquid chromatographic method previously described.<sup>10 14</sup> Samples were mixed with internal standards (turanose and  $\alpha$  methyl glucose), desalted with Zerolit DM-F resin, and dried under nitrogen at 50°C. They were then

derivatised with bis(trimethylsilyl)acetamide and trimethylchlorosilane before analysis on a 2.7 M column of 10% OV-17, operated at 245°C for lactulose and 200°C for mannitol estimation. Urine creatinine concentrations were measured using a Beckman Astra 8 analyser (Beckman RIIC).

Coefficients of variation between analyses were less than 10% for both markers. The Mann-Whitney U test was used to test the significance of the results.

## Results

We have expressed our results as urinary marker:marker and marker:creatinine excretion ratios calculated from the concentrations of lactulose, mannitol, and creatinine in serial daily urine samples. When the excretions of two markers, which are absorbed by different routes, are compared, the effects of rate of gastric emptying, intestinal transit, compartmental distribution, and renal clearance on the results apply to both equally, and therefore cancel each other out. Once a steady state of input and output has been reached the urinary excretion ratio of lactulose:mannitol represents a ratio of the absorption of these two markers. Work in this nursery has shown that daily urinary creatinine excretion remains approximately constant during the first two weeks of life in the preterm infant (Coulthard and Hey, unpublished data, 1982). Urinary marker:creatinine ratios were adjusted to account for the daily increase in the amounts of markers excreted because of increasing oral inputs and were compared with the steady urinary creatinine output by multiplying the marker creatinine ratios by the volume of feed taken on day one divided by the volume taken on the day in question.

Fig. 1 shows the median urinary lactulose:mannitol excretion ratio of all preterm babies studied from the first to the 7th day after starting oral test feeds. There is a daily decline in median excretion ratios with a significant difference between values on days one and four ( $P < 0.001$ ).

When the babies were divided into three groups according to gestational age, a similar decline in median urinary lactulose:mannitol excretion ratios was seen. Group 1 comprised 13 babies of less than 34 weeks' gestation at birth (mean gestation 30 weeks, range 27 to 33 weeks and mean birthweight 1535 g, range 1030 to 2180 g). Group 2 comprised 13 babies of 34 to 37 weeks' gestation at birth (mean gestation 35 weeks, mean birthweight 2173 g, range 1870 to 2910 g).

Figs. 2(a) and 3(a) show the urinary lactulose:mannitol excretion ratios on days one, four, and 7 after starting oral feeds, for each baby in groups 1 and 2. On some occasions a baby had been

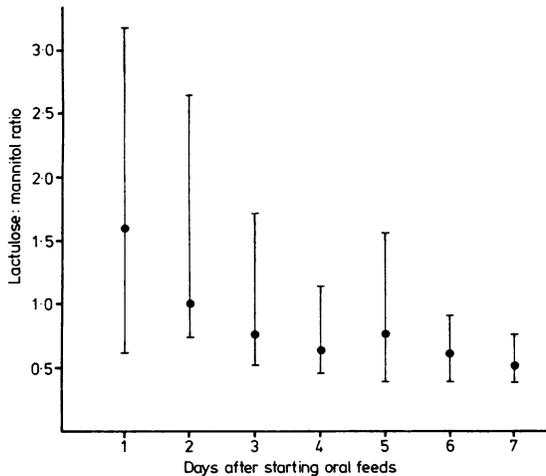


Fig. 1 Median urinary lactulose:mannitol excretion ratios (25th–75th centile).

discharged or transferred from the hospital, or a urine collection omitted, and no excretion ratio for a particular day was obtained. The more immature babies (group 1) displayed higher median urinary lactulose:mannitol excretion ratios on days one and four than the babies in group 2 ( $P<0.01$ ).

Expressing the results as urinary lactulose:creatinine and mannitol:creatinine ratios (Figs. 2(b) and (c), 3(b) and (c)) shows that this decline in urinary lactulose:mannitol excretion ratios was caused by decreased lactulose rather than increased mannitol absorption. A significant decline in median urinary lactulose:creatinine excretion ratios between days one and four was seen ( $P<0.01$ ) in group 1, and between days one and 7 ( $P<0.05$ ) in group 2. But none in the median urinary mannitol:creatinine excretion ratios in either group.

A group of 7 term babies (group 3) of greater than 37 weeks' gestation (mean gestation 40 weeks, mean birthweight 3507 g, range 2945 to 4360 g), showed no significant differences in urinary lactulose:mannitol, lactulose:creatinine, or mannitol:creatinine excretion ratios during the first four days after starting oral feeds (Table).

Table Urinary excretion ratios (median (range)) on days 1 to 4 after starting oral feeds in 7 term babies (group 3)

Excretion ratio	Day 1	Day 2	Day 3	Day 4
Lactulose:mannitol	0.56 (0.35–0.90)	0.56 (0.28–0.94)	0.57 (0.28–1.34)	0.59 (0.28–1.59)
Lactulose:creatinine	0.18 (0.10–0.26)	0.14 (0.09–0.16)	0.13 (0.07–0.34)	0.20 (0.10–0.62)
Mannitol:creatinine	0.32 (0.14–0.61)	0.18 (0.10–0.33)	0.20 (0.07–0.50)	0.35 (0.19–0.91)

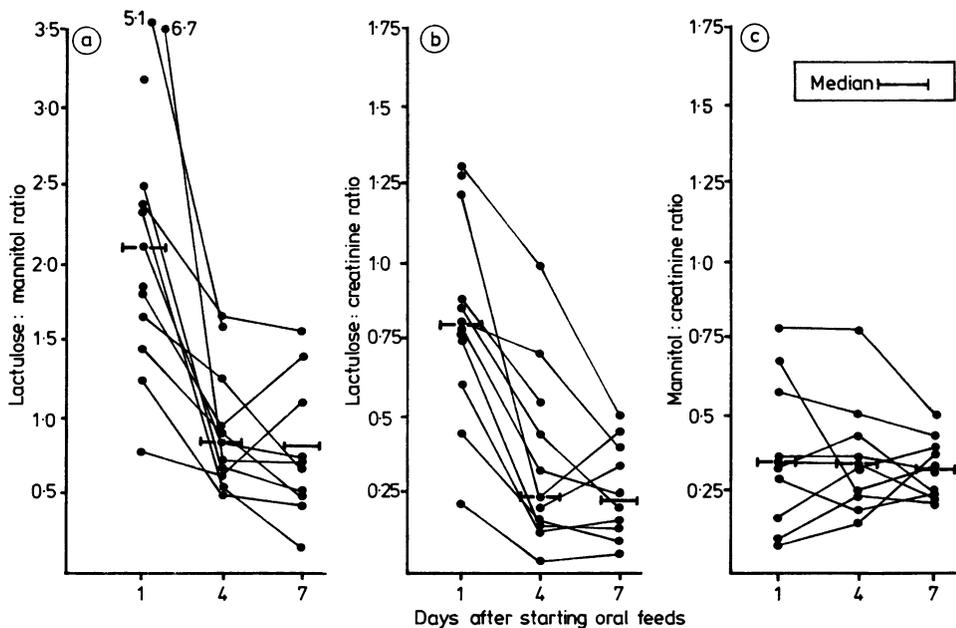


Fig. 2 Urinary excretion ratios in infants of less than 34 weeks' gestation (group 1).

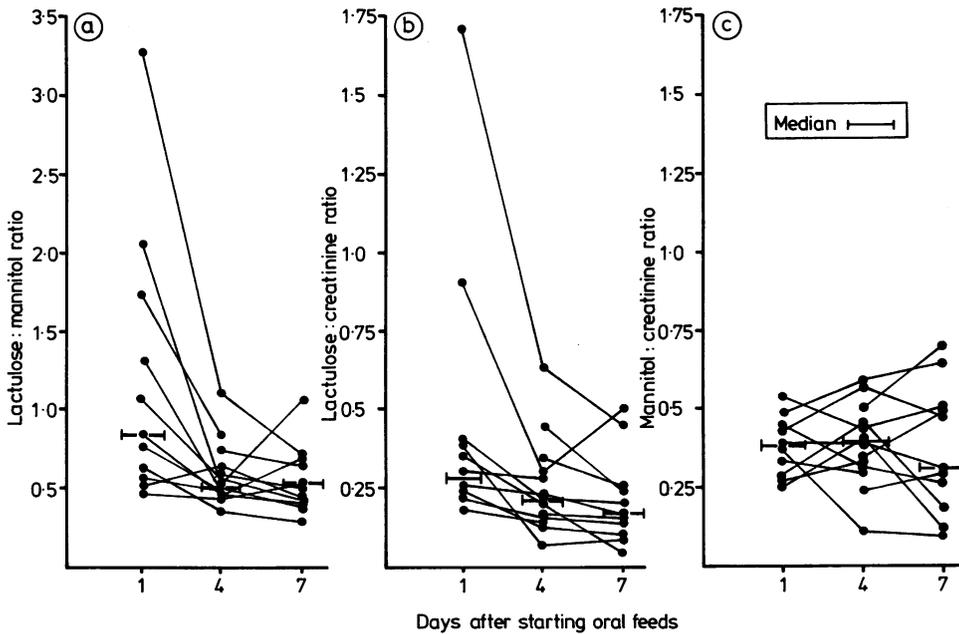


Fig. 3 Urinary excretion ratios in infants of between 34 and 37 weeks' gestation (group 2).

Those preterm babies of greater than 33 weeks' gestation at birth achieved urinary lactulose:mannitol excretion ratios comparable with term, formula fed babies within four days of starting oral feeds, as shown by the absence of a statistical difference between excretion ratios recorded on day four in the two groups.

Thirty five per cent of babies received their first oral feed within 24 hours of birth, 48% between 24 and 48 hours, and 17% after 48 hours. No relation between initial or subsequent urinary excretion ratios and time of onset of oral feeding, birthweight, assisted ventilation, prescription of antimicrobial drugs, or hyperbilirubinaemia was seen.

## Discussion

The different routes of passage across the intestinal wall proposed for the two markers may be explained by their molecular sizes. Lactulose (MW 342) has a molecular radius of 0.52 nm, and mannitol (MW 182) 0.40 nm. The permeability of the small intestine to small, water soluble substances seems to depend on molecular size; the adult intestine is penetrated by pathways of radius 0.3 to 0.9 nm with a higher population of the larger pathways in the jejunum than ileum.<sup>15</sup> Aqueous pores of radius 0.40 nm in the enterocyte cell membrane may allow the

passage of small molecules the size of mannitol, but exclude larger molecules. Pathways for the latter are probably represented by intercellular spaces where junctional complexes allow the free passage of molecules ranging in size from disaccharides to dextrans. Extrusion zones at villous tips and pinocytosis may also play a part in disaccharide and macromolecular uptake.

A tightening up of the junctional complexes,<sup>9</sup> and a loss of pinocytotic activity,<sup>16</sup> would lead to a reduction of lactulose absorption, but would have much less effect on mannitol absorption because of the continuing availability of the large population of aqueous pores by which only the smaller marker may pass. Any rise in this, caused by the increase in surface area which probably follows oral feeding,<sup>17</sup> might offset the portion of mannitol absorbed by paracellular rather than transcellular routes.

In preterm babies limitation of lactulose absorption seems to follow oral feeding. Does this pattern represent a positive mechanism designed to promote maximal intestinal uptake only during the initial period of enteral nutrition, or a functional immaturity of the gut in the preterm?

Our findings resemble the decline in protein absorption that follows oral feeding described in many animals.<sup>5</sup> Although lactulose is a much smaller molecule than most proteins, it may share the

same route of uptake. Those species that depend upon colostrum for their acquisition of immunoglobulins enjoy a period of enhanced macromolecular absorption followed by 'intestinal closure' during the neonatal period and this process is an essential part of extrauterine adaptation. Calves and piglets denied colostrum during this critical period are susceptible to bacterial enterocolitis and septicaemia. The human newborn acquires its immunoglobulins transplacentally before birth, and although colostrum is rich in secretory IgA, absorption of this immunoglobulin may not be important as its function is to act locally on the surface of the intestinal mucosa. One study, however, using anti-poliovirus IgA as a marker, showed measurable amounts of this immunoglobulin in the serum of term babies who received it within 24 hours of birth. None was detected in babies receiving it after this time.<sup>18</sup>

A greater intestinal permeability for a longer period would be an advantage to the preterm baby denied substantial prenatal transplacental acquisition of immunoglobulins. If a period of enhanced intestinal permeability after birth were, however, an essential part of postnatal adaptation to oral feeding, one would expect it to be present in the term as well as the preterm neonate; but this is not true for lactulose uptake. Preterm babies have also been shown to have higher serum concentrations of milk proteins than term babies fed equivalent artificial milk formulas,<sup>19</sup> suggesting that there is greater protein uptake from the intestine in the preterm baby. Even though food antigen absorption may persist during infancy,<sup>20</sup> these findings in man may well represent an immaturity of gastrointestinal function in the preterm infant rather than a process essential to extrauterine adaptation.

The gut of the preterm baby born before about 34 weeks' gestation is immature in many ways and a coordinated pattern of sucking and swallowing is not present in these babies.<sup>21</sup> The first feed of human milk taken by term babies after birth is associated with a rise in blood glucose, plasma insulin, and other gastrointestinal hormone concentrations, but there is no change in the circulating values of any of these metabolites or hormones after the first feed of babies of less than 34 weeks' gestation. Within three days of birth, however, preterm infants show a response comparable with term babies.<sup>22</sup> The intestinal lactase activity of babies born before 34 weeks' gestation is only 30% of that of term babies, but is followed by a rapid rise to mature values.<sup>23</sup> The ability to mount a detectable immune response to ingested food antigens is not acquired until about 35 weeks' gestation.<sup>24</sup> D-glucose and D-xylose absorption are appreciably lower in preterm than term babies, and there is a postnatal surge in active

D-glucose absorption at about 7 days after birth in babies born before 32 weeks' gestation.<sup>25</sup> These findings all suggest that the gastrointestinal tract of babies born before about 34 weeks' gestation is not fully prepared for enteral nutrition. Our results support this, although the steep and rapid decline in lactulose permeability we have shown and the other postnatal changes described above indicate that considerable and vigorous adaptation to enteral nutrition can take place in the preterm baby after the onset of oral feeding.

We suggest that the enhanced intestinal permeability of the preterm baby to lactulose represents an immaturity of the gut mucosa and that the rapid decline in permeability that follows the onset of oral feeding is an adaptation aimed at achieving mature intestinal function as soon as possible. The physiological mechanisms and morphological changes in this process remain unknown. Whether an enhanced intestinal permeability during the immediate neonatal period is also an advantage to the newborn baby should be investigated by studying the permeability of the gut of the term baby to immunoglobulins during the period when oral feeding begins.

We should like to thank the staff of the Princess Mary Maternity Hospital for their enormous help, Dr E J Eastham for his advice, and Wyeth and Milupa who provided the infant milks used. This study was supported by the Newcastle Area Health Authority.

#### References

- Grand RJ, Watkins JB, Torti FM. Development of the human gastrointestinal tract. *Gastroenterology* 1976;**70**:790-810.
- Aynsley-Green A. The control of the adaptation to postnatal nutrition. In: Zoppi G, ed. *Metabolic-endocrine responses to food intake in infancy*. Basel: Karger, 1982:59-87.
- Bickel H. Mellituria, a paper chromatographic study. *J Paediatr* 1961;**59**:641-56.
- Rothberg RM. Immunoglobulin and specific antibody synthesis during the first weeks of life of premature infants. *J Pediatr* 1969;**75**:391-9.
- Walker WA. Gastrointestinal host defence: importance of gut closure in control of macromolecular transport. In Elliott KM, Whelan J, eds. *Development of mammalian absorptive processes*. Amsterdam: Excerpta Medica, 1979:201-19.
- Beach RG, Menzies IS, Clayden GS, Scopes JW. Gastrointestinal permeability changes in the preterm neonates. *Arch Dis Child* 1982;**57**:141-5.
- Beach RC, Menzies IS. Lactulose and other non-absorbable sugars in infant milk feeds. *Lancet* 1983;**i**:425-6.
- Dahlqvist A, Gryboski JD. Inability of the human small-intestinal lactase and hydrolyse lactulose. *Biochim Biophys Acta* 1965;**110**:635-6.
- Laker MF, Menzies IS. Increase in human intestinal permeability following ingestion of hypertonic solutions. *J Physiol* 1977;**265**:881-94.
- Laker MF, Bull HJ, Menzies IS. Evaluation of mannitol for use as a probe marker of gastrointestinal permeability in man. *Eur J Clin Invest* 1982;**12**:485-91.
- Menzies IS. Absorption of intact oligosaccharide in health and disease. *Biochem Soc Trans* 1974;**2**:1042-7.

- <sup>12</sup> Saunders DR, Wiggins HS. Conservation of mannitol, lactulose, and raffinose by the human colon. *Am J Physiol* 1981;**241**:G397-402.
- <sup>13</sup> Parkin JM, Hey EN, Clowes JS. Rapid assessment of gestational age at birth. *Arch Dis Child* 1976;**51**:259-63.
- <sup>14</sup> Laker MF. Estimation of disaccharides in plasma and urine by gas-liquid chromatography. *J Chromatogr* 1979;**163**:9-18.
- <sup>15</sup> Fordtran JS, Rector FC, Ewton MF, Soter N, Kinney J. Permeability characteristics of the small intestine. *J Clin Invest* 1965;**44**:1935-44.
- <sup>16</sup> Lecce JG, Broughton CW. Cessation of uptake of macromolecules by neonatal guinea pig, hamster, and rabbit intestinal epithelium (closure) and transport into blood. *J Nutr* 1974;**103**:744-50.
- <sup>17</sup> Widdowson EM, Colombo VE, Artavanis CA. Changes in the organs of pigs in response to feeding for the first 24 hr after birth. *Biol Neonate* 1976;**28**:272-81.
- <sup>18</sup> Ogra SS, Weintraub D, Ogra PL. Immunologic aspects of human colostrum and milk. *J Immunol* 1977;**119**:245-8.
- <sup>19</sup> Robertson DH, Paganelli R, Dinwiddie R, Levinsky RJ. Milk antigen absorption in the preterm and term neonate. *Arch Dis Child* 1982;**57**:369-72.
- <sup>20</sup> Easthan EJ, Lichauro T, Grady MI, Walker WA. Antigenicity of infant formulas: role of immature intestine on protein permeability. *J Pediatr* 1978;**93**:561-4.
- <sup>21</sup> Gryboski JD. The swallowing mechanism of the neonate 1. Esophageal and gastric motility. *Pediatrics* 1965;**35**:445-52.
- <sup>22</sup> Lucas A, Bloom SR, Aynsley-Green A. Metabolic and endocrine events at the time of the first feed of human milk in preterm and term infants. *Arch Dis Child* 1978;**53**:731-6.
- <sup>23</sup> Antonowicz I, Lebenthal E. Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. *Gastroenterology* 1977;**72**:1299-303.
- <sup>24</sup> Rieger CHL, Rothberg RM. Development of the capacity to produce specific antibody to an ingested food antigen in the premature infant. *J Pediatr* 1975;**87**:515-8.
- <sup>25</sup> McNeish AS, Ducker DA, Warren IF, Davies DP, Harran MJ, Hughes CA. The influence of age and size on the absorption of D-xylose and D-glucose from the small intestine of the human neonate. In: Elliott KM, Whelan J, eds. *Development of mammalian absorptive processes*. Amsterdam: Excerpta Medica, 1979:267-80.

Correspondence to Dr L T Weaver, Department of Paediatrics, The Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP.

Received 5 December 1983