Nutrient Absorption in the Developing Colon

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The function of the adult colon has been well defined and thoroughly studied and includes the transport of salt and water from the colonic lumen to the circulation in a process that is responsive to aldosterone (1–3) and contributes a large portion of normal body salt and water conservation. Only after the greatest fraction of the mass of ingested and secreted intestinal fluid has been recovered in the colon is it possible for the kidneys to perform the “fine-tuning” adjustments to the fluid and electrolyte status of the body that maintain homeostasis, a fact made apparent in the rapid and severe dehydration that will ensue in the patient who has lost his colon for whatever reason and then acquires what would be an otherwise mild diarrhea in the healthy population.

Other functions were once attributed to the adult colon, not surprisingly, those of nutrition and the absorption of nutrients such as sugars and proteins (4). Although, as we shall see, this attribution is not uniformly in error, there have been many experiments performed in diverse animals, and with the exception of relatively small amounts of sugars absorbed from the colons of two species (5), it is safe to assume that sugars and amino acids placed in contact with the adult colonic epithelium will not be absorbed in significant amounts (6–12). Just how well these assumptions regarding colonic function might apply to the newborn human or any other newborn animal and, even more importantly for medicine, to the premature infant has been almost totally unknown until recently. Advances in the care of the premature infant have been based on solid understanding of the normal development of physiological function including cardiovascular, pulmonary, and endocrine development. The developmental physiology of the intestinal tract, however, particularly the colon, has been relatively neglected, and yet there is an interesting story to be learned from watching its development.

The adult colon is covered by an epithelium consisting of a single layer of columnar cells arranged in a flat sheet, with interspersed crypts at regular intervals along the mucosa. The fetal and neonatal colon differs considerably from this pattern, however, both in the human and in the much more often studied rat. In very early studies of the human embryo (13), it was realized that the colon differed from the adult in the embryo by the presence of villi, which make their appearance in the 58-mm crown–rump length specimen (approximately 12 weeks gestation). This is accompanied by a complex process of development
which transforms a primitive stratified epithelium into a columnar epithelium, which more closely resembles that in the adult (14–16). The villi remain through the second trimester and are lost during the third.

In the rat, a similar process occurs (17), but only in the postnatal period are the villi of the colon lost (18–23). The rat, which is born at the 22nd day of gestation, has only a tiny lumen at 16 days of gestation, and this is surrounded by a stratified layer of undifferentiated epithelium two to three cell layers thick. On the 17th day, the epithelium is still stratified but has increased to three to five cell layers thick. On the 18th day, the colon develops rudimentary crypts, and by the 20th day, goblet cells are present, and the epithelium is single-cell columnar (17). This maturation is accompanied by increasing synthesis of glycoproteins, an adult function of colonocytes. Incorporation of $^3$H-galactose and $^3$H-glucose into glycoproteins and the assumption of adult patterns of intracellular migration are seen during the last 4 days of gestation (24). These columnar cells have an apical tubulovesicular system which is associated with the absorption of intact proteins, including immunoglobulins, in the small intestine, and indeed they have been seen in the fetal and neonatal colon as well (19,25). The colon maintains this peculiar anatomic and functional appearance until the ninth to 10th day after birth in the rat, when the villi are lost by a process of constriction at their bases that eventually leaves the adult-type flat epithelium (23).

A surprising early study showed that the newborn rat colon contains alkaline phosphatase (26). That the anatomic differences from adult in the fetal colon may have correlations in the ability of the colon to transport nutrients is suggested by the presence of lactase in the neonatal rat colon (19) but not in that of the newborn pig (27). Carbonic anhydrase and acid phosphatase are also present at birth (19). The alkaline phosphatase activity is confined to the cecum and proximal colon during the first 2 weeks of postnatal life and then disappears (19,21). The distal colon has some alkaline phosphatase activity initially, but this is lost by the third postnatal day (19,21). Alkaline phosphatase, along with $\beta$-glutamyl transpeptidase, has recently been shown to be present in the pig at birth, and both are lost during the first few days of postnatal development (27). Activity of $\beta$-naphthylaminidase, a peptidase, was also present in the proximal colon of the newborn pig (27).

Studies of enzyme levels have also been performed in the fetal human intestinal tract, and when the activities of L-alanyl-L-glutamic acid, L-alanyl-L-proline, glycyglycine, glycyl-L-leucine, and glycyl-L-valine dipeptidases were estimated in fetuses between 11 and 23 weeks of gestation, it was discovered that their activities were quite similar along the entire length of the intestine including the colon (28). Thus, the presence of enzymes capable of digesting dipeptides is established long before birth and is present in an organ not ordinarily associated with any digestive function at all. Similar studies have been done on the disaccharidases and alkaline phosphatase activities of the human fetal intestine, specifically looking at maltase, invertase, isomaltase, lactase, cellobiase, trehalase, and alkaline phosphatase (29). Whereas in the colon the dipeptidase levels were the same as in the small intestine, in the case of these disaccharidases the
activities are on the order of half of the jejunal levels. Precisely when these activities first appear and when they are lost are not known, nor is there available a good picture of what factors may influence their induction and disappearance.

The actual uptake of amino acids and glucose in the developing intestine has been the subject of much prior investigation, and some of these studies have included the colon. The cecum of the chicken begins to accumulate α-methylglucoside at about the time of hatching; the accumulation gradient increases during the first week and is then lost quickly after the first week after hatching (30). In the rat, the proximal colon is capable of 3-O-methylglucose tissue accumulation on the first day after birth to a level about one-third as great as that of the ileum at the same age (31). This ability is then no longer present at 32 days of age, but the time of its loss has not been obtained.

That the absorption of lipids from the fetal colon may occur is apparent from the formation of lipid droplets in the fed neonatal pig proximal colon (32) and in the fetal human colon (15).

The absorption of macromolecules is demonstrable in the small intestine of the fetal rat when horseradish peroxidase is injected into amniotic fluid and the fetus is allowed to swallow the fluid before electron micrographs of the intestine are prepared (33). The absorption of the macromolecule was not demonstrated in the colon of the fetal rats but has been demonstrated in suckling rats with the horseradish peroxidase technique by at least two investigators (19,25). The absorption seems to be limited to the proximal colon only. Another form of confirmation of macromolecule transport comes from the induction of hypoglycemia by the administration of insulin enemas to the neonatal rat, an effect that is lost after 4 weeks (19).

Calcium absorption has also been studied to some extent and appears to proceed by a carrier-mediated process in the adolescent rat but by passive diffusion in the suckling colon when each is perfused via an in vivo intraluminal technique (34). Tissue accumulation also occurs in the colon early in life (rat) but decreases in the colon while increasing in the duodenum (31).

There is a large amount of information concerning factors important in the development of small intestinal functions, and this has been reviewed (35,36). There has been very little work on the colon in this respect. Jarvis et al., in a report on cell replacement, mention that in unpublished work they found that if parturition was advanced with prostaglandin analogs or retarded with progesterone in the pig, there was no observable effect on the ability of the mucosa to concentrate methionine during the period of study (37). Studies from the same institution show that ileostomy delays the decrement in methionine transport that usually occurs in the pig after birth but not the development of amiloride-sensitive Na⁺ transport (38). This would imply that the presence of the normal fecal content somehow accelerates the loss of fetal enterocytes and their replacement by the suckling type of enterocyte (38). Finally, recent work has shown that methylprednisolone can prematurely suppress the absorption of calcium and magnesium from the colon of the suckling rat (39). It has no effect on net absorption of either from the adolescent rat (39).
Although a more direct look at the transepithelial transport of sugars, amino acids, and ions would be desirable, there are many difficulties inherent in such studies. Human tissue is seldom available and, when available, is often in poor condition. Large animals are expensive and cumbersome to maintain, often with long gestation periods. The laboratory rat is easily available and ideal in many respects, since its intestine is still very immature by comparison to the human until just a few days before birth, but the minute size of the intestine and colon has been an obvious impediment to its study, since such small tissues are not amenable to typical methods utilizing half-chambers that are applied to adult tissue.

Accordingly, based on the results published in the available literature, we began a study of absorptive function in fetal and neonatal rat gut (39a). Initially, we looked at small bowel absorptive function and ultimately applied similar techniques to the colon. We constructed a system, adapted from the methods for determination of transport in individual segments of the nephron, that allowed the in vitro luminal perfusion of segments of the intestine from rat fetuses as early as 19 days of gestation.

We used Sprague-Dawley rats of known gestational age to obtain fetuses on the 19th or 20th day of a 22-day gestation. Ether was used as an anesthetic (40), and after maternal laparotomy, the dissection of the fetus was carried out on the stage of a stereomicroscope. A 1-cm length of the gut was then isolated in a heated dish containing oxygenated saline, where it was pulled onto pipettes held in micromanipulators and tied in place with 7-0 silk suture.

To measure transport, the solute desired was dissolved in a buffered saline perfusion solution along with polyethylene glycol, average molecular weight 4,000 (PEG), which served as a nonabsorbable marker. The perfusate flowed from a syringe pump at 1.42 μl/min and, after traversing the segment of gut, was collected from a pipette at the opposite end for analysis. In some experiments a double-lumen pipette was used to allow for rapid changes from one perfusate to another during an experiment. Also, in some experiments a digital electrometer was connected through agar bridges to allow measurement of the electrical potential difference across the epithelium.

The perfusate was a standard buffered saline solution which has a pH of 7.4 when bubbled with 95% oxygen, 5% CO₂; ³H-PEG was added to the perfusate, and glucose, 3-O-methyl glucose (3-O-MG), or L-alanine was also added to the perfusate or bath as desired to measure absorption. The suitability of ³H-PEG as a nonabsorbable marker, which is known in the adult intestine (41), was confirmed in the fetal intestine, and absorption was calculated according to standard formulas.

Glucose was absorbed from the ileum of the 19-day fetus at 200 ± 17 μmole·hr⁻¹·g⁻¹ (n = 7), and the rate of absorption increased to 378 ± 12 μmole·hr⁻¹·g⁻¹ (n = 7) in the 20-day fetuses (p < 0.01). Both determinations were made with 10 mM glucose in the bath and the perfusate. In addition, this absorption of glucose was found to be stable for at least 1 hr before decremental changes in rate of absorption occurred. Since the absorption of glucose may
represent glucose that is both transported across the epithelium and metabolized by the epithelium, we were interested in studying the result obtained with the perfusion of 3-O-MG, which is transported by the same mechanisms as glucose but not metabolized (42). For these experiments, either the bath or the perfusate contained 10 mM 3-O-MG sequentially, so that the unidirectional flux rate from lumen to bath or from bath to lumen could be determined. In the case of lumen to bath, the flux was $88 \pm 10 \mu\text{mole}\cdot\text{hr}^{-1}\cdot\text{g}^{-1} (n = 5)$ at 19 days of gestation and almost doubled to $160 \pm 16 \mu\text{mole}\cdot\text{hr}^{-1}\cdot\text{g}^{-1} (n = 5)$ at 20 days of gestation ($p < 0.01$). The bath-to-lumen flux rate was small, $-17 \pm 2 \mu\text{mole}\cdot\text{hr}^{-1}\cdot\text{g}^{-1} (n = 7)$ and did not differ between the two age groups.

If the absorption of glucose proceeds by the active transport mechanisms seen in adult tissue, it should depend on the presence of Na$^+$ (43), and so experiments were done in which Na$^+$ was replaced with choline. Under these conditions, and with 10 mM glucose in the perfusate, the absorption of glucose was $-3 \pm 16 \mu\text{mole}\cdot\text{hr}^{-1}\cdot\text{g}^{-1} (n = 7)$, a value not significantly different from zero ($p > 0.5$).

A perfused tubular epithelium should demonstrate flow dependence of transport (44). That is, the transport rate should depend on the mean luminal concentration of substrate, and the concentration should in turn be dependent on the flow rate of the perfusate being extracted from the lumen. In the perfused 20-day ileum, this effect was observed when the flow rate of the perfusate containing glucose was increased from $1.42 \mu\text{l}\cdot\text{min}^{-1}$ to $3.52 \mu\text{l}\cdot\text{min}^{-1}$. There was a corresponding increase in absorption of glucose (10 mM) to $513 \pm 32 \mu\text{mole}\cdot\text{hr}^{-1}\cdot\text{g}^{-1} (n = 6) (p < 0.01)$. This flow dependence was further evidence for the in vitro function of the perfused intestine.

We next addressed the possibility that the in vitro perfused colon of the 20-day fetus would absorb glucose, since the histological appearance of the colon matures about 24 hr behind the small intestine (17). Under conditions very similar to those in the intestine, we have discovered that the colon absorbs glucose at approximately half the rate of the intestine. Unidirectional flux rates for 3-O-methylglucose, a nonmetabolized but transported glucose analog, confirmed that this absorption of glucose represents actual transport, and in experiments in which sodium is removed from both the bath and perfusate the absorption of glucose is essentially abolished.

The colon also appears to absorb L-alanine in these experiments and does so at a rate nearly equal to that for glucose. The transport of L-alanine is partially but not completely inhibited by the absence of sodium into perfusate and bath.

There is a significant base-line flux of sodium from the lumen to the bath accompanied by a lumen-negative potential difference in the 20-day gestation colon. The sudden addition of glucose to the perfusate causes a significant increase in the outward flux of sodium and in the negativity of the lumen. This is entirely consistent with the idea that glucose is being cotransported with sodium in an electrogenic mechanism.

This work has established that the fetal intestinal tissues, including colon, can function in this in vitro system for periods in excess of 2 hr without significant
decrement in fluxes of several solutes. It is of interest that the transport of glucose is present on the day the epithelium is first known to achieve a villus architecture (45) and that it doubles over the next 24 hr. That such a distinct uptake of glucose does not merely represent its uptake and metabolism by the tissue itself rather than its transepithelial transport is suggested by the fluxes of 3-O-MG, which proceed in roughly the same proportion to glucose in the fetal intestine as in the adult (18). As could be expected, there is evidence to indicate that the active transport of glucose occurs by the same mechanism as in the adult, at least in that it is sodium dependent, since all of the transport of this solute was abolished in the absence of sodium.

The surprising finding about the fetal colon is its ability to actively transport sugars and amino acids. Previous work in animals and in humans failed to demonstrate any such function for the colon, but there is at least one exception. The adult colon in the dog has limited capacity to absorb glucose, and it may be that rather than a complete absence of glucose transport it is appropriate to think of the colon in terms of a sharply qualitatively reduced capacity.

The ability of the fetal and newborn colon to transport nutrient solutes is apparently widespread, however, being present to some degree in chicken (30), rat (31), pig (37,37a), and lamb (46). As just shown, this capacity extends to at least one amino acid in the rat and to many others in the newborn sheep. We have just seen that transport of glucose in the fetal rat colon is indeed transepithelial, that it increases the transport of Na⁺, and that it produces an increase in the negativity of the luminal potential. All are strong indications that the colon must be, at the least, capable of an important role in nutrient absorption.

The significance of this unusual extension of roles in the colon must remain speculative at the present. We know that the more thoroughly studied amino acid methionine causes an abrupt increase in short-circuit current and, correspondingly, in net Na⁺ transport in the newborn pig (47). This results in depolarization of the microvillous membrane, probably the consequence of electrogenic Na⁺ influx into the cell (48). The electrogenic influx of Na⁺ associated with adult colon, i.e., that which is not associated with the presence of glucose or amino acids and which is blocked by amiloride, does not appear until later (49).

These findings have given rise to speculation that the colon may serve as an extension of the function of the small bowel at a time when the postnatal absorption of intact immunoglobulins has been shown to temporarily interrupt normal small intestinal absorption of Na⁺ (50). The nonionic solute-associated Na⁺ uptake occurring in the colon may thus serve to increase both Na⁺ and nutrient absorption at a critical point in development.

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REFERENCES


8. Crane RK, Mandelstam P. The active transport of sugars by various preparations of hamster intestine. Biochim Biophys Acta (1960);45:460-76.

9. Davidson JN, Garry RC. The absorption of monosaccharides from the large intestine of the rat under urethane anesthesia. J Physiol (Lond) (1939);96:172-5.


42. Goldner AM, Schultz SG, Curran PF. Sodium and sugar fluxes across the mucosal border of rabbit ileum. J Gen Physiol (1969);53:362-83.
47. James PS, Smith MW. Methionine transport by pig colonic mucosa measured during early post-natal development. J Physiol (Lond) (1976);262:151-68.