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Amino acid absorption in the mammalian colon

Although electrolytes are actively absorbed and secreted by the mammalian large intestine[1–3], active transport of both amino acids and monosaccharides has not been demonstrated in the colon[4–6]. The absence of active amino acid transport in the colon does not exclude colonic absorption. Therefore, this study was directed to ascertain whether movement of amino acids across colonic mucosa does occur, and, if so, by what mechanism.

Experiments were performed utilizing several parameters to study absorption in adult male Sprague–Dawley rats and adult male New Zealand rabbits.

In vivo experiments: 2 ml of a 10 mM L-alanine solution were introduced into a cleansed colon of an anaesthesized rat and after 30 min the colon was removed, drained and weighed. L-Alanine absorption was determined after correction of water absorption by measurement of polyethylene glycol and expressed as μmoles per 30 min per g wet tissue weight.

In vitro experiments: L-Alanine transport in the rat was studied using standard methods of everted gut sacs[7] and rings[8]. In the studies utilizing everted gut sacs, 1 ml of a Krebs–bicarbonate buffer was placed inside the sac which was then placed in 5 ml of buffer solution containing 0.01–25 mM L-alanine and approx. 0.3 μC L-[^{14}C]alanine. In certain experiments the serosal fluid contained buffer only, and in other studies the serosal media had the same concentration of L-alanine as the mucosal solution. The flasks were gassed with O_2–CO_2 (95:5, v/v) and incubated at 37° for 30 min. Detailed studies of L-alanine accumulation were performed utilizing intestinal rings[8]. Everted segments of colon were sliced and three rings weighing approx. 30 mg each were placed in 2 ml of buffer solution containing L-[^{14}C]alanine. The tissue was incubated at 37° after gassing with O_2–CO_2 (95:5, v/v). The rings were weighed and placed in boiling water for 10 min. Radioactivity of both the incubation media and the water solution was determined. Extracellular space was determined with [^{14}C]-inulin and was corrected for in all calculations. The colonic tissue was heated at 110° overnight and dry tissue weight was obtained. Accumulation of L-alanine was expressed by comparing the concentration of L-alanine in the intracellular fluid compared to that in the incubation media. In studies of potential inhibition, equal numbers of control rings were used simultaneously, and the results are expressed as a percent of control values. In studies with increasing substrate concentration, results are expressed as L-alanine accumulation in intracellular fluid per g wet tissue weight.

Studies of glycine influx were performed in large intestinal mucosa from rabbits utilizing the method and apparatus described by SCHULTZ *et al.*[9]. Glycine influx was determined after a 120-sec incubation; preliminary studies demonstrated that influx was linear for at least 240 sec. Influx of 5 mM glycine was determined in the presence and absence of Na^+, 30 mM L-leucine and 30 mM L-proline. Glycine influx was also measured at several concentrations between 7 and 280 mM.

Evidence of active transport could not be demonstrated either by the everted gut sac method or by the ring method. Following incubation of everted sacs whose mucosal and serosal fluid contained equal concentrations of L-alanine, the ratio of mucosal to serosal concentration of L-alanine was always less than 1. L-Alanine concentrations in these experiments were 0.01, 1, 10, and 25 mM.

In ring experiments with incubation periods of 1–60 min at L-alanine concentrations of 0.01, 10, 25 or 100 mM, an accumulation of L-alanine in the intracellular fluid greater than that in the incubation media was never observed. Under the conditions of these studies accumulation against a concentration gradient was not demonstrated.

However, in everted gut sac experiments in which L-alanine was placed only in the mucosal media, the amino acid appeared in the serosal fluid. 1.71 ± 0.68 μmoles L-alanine per g wet tissue weight were transported to the serosal fluid in nine sacs from three rats.

In *in vivo* studies in which 10 mM L-alanine was placed *in situ* in the colon for 30 min, 9.13 ± 1.48 μmoles of L-alanine per g wet tissue weight disappeared from the lumen of the colon after correction for fluid movement.

Detailed studies of L-alanine transport were performed with segments of colonic mucosa. Accumulation occurred rapidly approaching maximal values within 5 min. Therefore, 1-min incubation periods were used in subsequent studies. No evidence of saturation was observed. There were increased amounts of L-alanine in the tissue water with increasing concentrations of L-alanine in the incubation media (Fig. 1). Decrease in accumulation could not be demonstrated by methods which normally inhibit active amino acid transport in the small intestine (Table I). Incubation in the presence of other neutral amino acids, 10 mM L-methionine, 10 mM L-leucine, 10 mM L-phenylalanine and 10 mM glycine also did not inhibit the uptake of 1 mM L-alanine.

TABLE I

ACCUMULATION OF 10 mM L-ALANINE BY RAT COLON RINGS

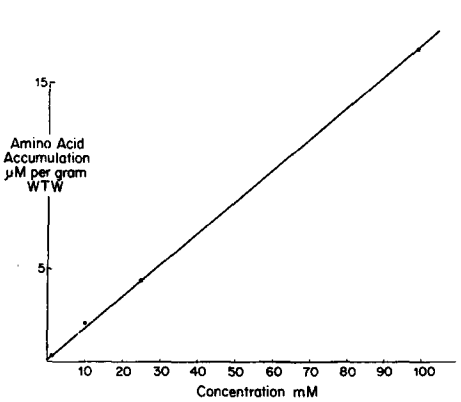
Rat colon rings were prepared as described in text. The incubation period was 1 min at 37° in an O_2–CO_2 (95:5, v/v) atmosphere except as noted. The differences observed between experiments and paired control studies were not significant.

Environment	*Addition*	% *Accumulation of control*
N_2 at 37°		+ 3%
O_2–CO_2 (95:5, v/v) at 25°		+19%
O_2–CO_2 (95:5, v/v) at 37°	0.1 mM 2,4-dinitrophenol	− 8%
O_2–CO_2 (95:5, v/v) at 37°	0.1 mM ouabain	+11%
O_2–CO_2 (95:5, v/v) at 37°	5 mM ethacrynic acid	0

The conclusions of the results of the glycine influx experiments are similar to the L-alanine studies. No inhibition of the influx of 5 mM glycine was observed by removal of sodium from the incubation media or by the addition of either 30 mM L-leucine or 30 mM L-proline. Increasing glycine concentrations from 7 to 280 mM in Na^+-free media resulted in a linear increase of glycine influx (Fig. 2).

These studies confirm earlier investigations that active transport of neutral amino acids does not occur in the mammalian colon[4,5]. This investigation does demonstrate that L-alanine crosses the everted colonic mucosa, disappears from the lumen of the large intestine, and enters the colonic mucosa but not against a concentration gradient.

The absorption of amino acids and other nonelectrolytes from the large intestine has been disputed and poorly substantiated. Although no evidence of active transport of amino acids exists in the mammalian colon, active transport, *i.e.* movement against a concentration gradient, frequently is not measured during most *in vivo* studies in which luminal concentrations are usually much greater than plasma levels. Under these conditions there is conflicting evidence whether absorption of any nonelectrolyte occurs[10–16]. Although L-alanine will not move against a concentration gradient, it can be transferred across rat colonic mucosa down a concentration gradient.



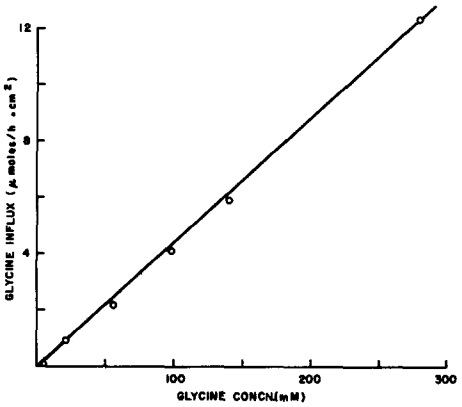


Fig. 1. Increased accumulation of L-alanine in the rat colon accompanied increase of initial concentrations of L-alanine in incubation media.

Fig. 2. Mucosal influx of glycine in the rabbit colon as a function of glycine concentration in the mucosal solution.

Detailed examination of amino acid accumulation failed to reveal evidence of either active transport or facilitated diffusion in the rat colon. In the ring experiments an accumulation of L-alanine of less than 1 was always observed. Accumulation could not be inhibited by maneuvers that interfere with active transport: an anaerobic environment, low temperature, addition of 2,4-dinitrophenol, ouabain or ethacrynic acid. A nonsaturable system was observed. Increased accumulation paralleled increased initial substrate concentrations (Fig. 1). Facilitated diffusion is characterized by a saturated system, a high Q_{10} and evidence of competitive inhibition. Accumulation was similar at both 25° or 37°. Other neutral amino acids, L-methionine, L-leucine, L-phenylalanine and glycine, which usually competitively inhibit L-alanine transport in the small intestine, did not alter L-alanine movement to any significant extent in the large intestine. The studies of the influx of glycine into the colonic mucosa of the rabbit produced similar results. No evidence of a carrier mediated system was observed.

As early as 1913 SHORT AND BYWATERS[17] demonstrated after the introduction of amino acids but not peptones into the human rectum that there was increased urinary nitrogen excretion which was believed evidence of intestinal absorption. 25 years later the disappearance of casein acid hydrolysates was observed in Thiry loops of dog colon[16]. More recent studies failed to demonstrate the movement of several amino acids, including L-tyrosine[4], L-monoiodotyrosine[18], L-alanine[5], L-histidine[5], L-glutamic acid[5], glycine[5] and L-aminoisobutyric acid[19], against a chemical gradient.

CHRISTENSEN *et al.*[19] demonstrated that both L-aminoisobutyric acid and cycloleucine are secreted into the colon but that maintenance of a plasma-lumen gradient did not occur[19]. BAILLIEN AND SCHOFFENIELS[5] observed minimal permeability across the colon of the Greek tortoise of L-alanine although active transport was not found. HOLDSWORTH AND WILSON[20] observed accumulation of glycine by the cecum of neonatal chickens, however, the cecal epithelium of these chicks resembles the small intestine histologically and ultrastructurally. Older chicks, whose cecum have changed histologically, no longer are able to accumulate glycine.

This investigation confirms the absence of an active transport system for amino acids in the large intestine. However, some transfer does occur and its mechanism is a nonsaturable, non-energy-dependent process, most likely diffusion.

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