Macromolecular transport across the rabbit proximal and distal colon

J A Hardin, M H Kimm, M Wirasinghe, D G Gall

Abstract

***Background*—Although many studies have investigated macromolecular uptake in the stomach and small intestine, little is known about macromolecular uptake in the colon.**

***Aims*—To investigate the mechanisms involved in the transport of large antigenically intact macromolecules across the proximal and distal colonic epithelium in the rabbit.**

***Methods*—The mucosal to serosal movement of bovine serum albumin (BSA) was examined in modified Ussing chambers under short circuited conditions. The mucosal surface was exposed to varying concentrations of BSA, and after a 50 minute equilibration period, the mucosal to serosal flux of immunologically intact BSA was determined by ELISA. Total BSA flux was determined by the transport of radiolabelled ^{125}I-BSA.**

***Results*—Intact BSA transport in proximal and distal colonic tissue showed saturable kinetics. Intact BSA transport in the proximal and distal segment was 7% and 2% of the total ^{125}I-BSA flux respectively. Immunologically intact BSA transport in the distal segment was significantly less than that in the proximal segment. Intact BSA transport in the proximal colon was significantly reduced following treatment with sodium fluoride, colchicine, and tetrodotoxin. Cholinergic blockade had no effect on the uptake of intact BSA.**

***Conclusion*—The findings indicate that the transport of intact macromolecules across the proximal and distal large intestine is a saturable process. Further, intact BSA transport in the proximal colon is an energy dependent process that utilises microtubules and is regulated by the enteric nervous system.**

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Gastrointestinal Research Group, University of Calgary, Alberta, Canada
J A Hardin
M H Kimm
M Wirasinghe
D G Gall

Correspondence to:
Dr D G Gall, Faculty of Medicine, Health Sciences Centre, 3330 Hospital Drive NW, Calgary, Alberta T2N 4Nl, Canada.

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The lumen of the intestine contains a variety of macromolecules, of which a small, nutritionally insignificant, proportion are transported across the mucosa immunologically intact.[1] A number of studies have described the existence of pathways for the uptake of intact macromolecules from the lumen across the mucosa of the stomach[2 3] and small intestine.[4–8] Macromolecular uptake in the upper gastrointestinal tract is a highly regulated phenomenon. In the stomach the uptake of intact protein involves an active, energy dependent mechanism that utilises the microtubular network[2] and is regulated by cyclooxygenase metabolites.[3] In the jejunum, mucosal to serosal macromolecular transport is a saturable process that is both microtubule and energy dependent[4 7] and is regulated by the enteric nervous system acting via muscarinic pathways.[7] Nitric oxide has also been shown to play a role in the regulation of jejunal macromolecular uptake.[6] Likewise, in the ileum, mucosal to serosal macromolecular uptake has been shown to be both microtubule and energy dependent[9] and under neural regulation acting through muscarinic pathways.[10] Furthermore, experiments using the adenylate cyclase activator forskolin suggest that cAMP plays a role in the regulation of ileal macromolecular uptake.[10] The transport of intact proteins in the small intestine also occurs in the serosal to mucosal direction.[4 11] As shown for mucosal to serosal uptake, jejunal serosal to mucosal macromolecular transport is both saturable, and energy and microtubule dependent.[11] However, unlike mucosal to serosal uptake, the serosal to mucosal flux of intact protein is not under neural control.[11]

Much less is known about macromolecular uptake in the colon. Studies have demonstrated the in vivo uptake of intact human growth hormone across rat colon[12] and bioactive arginine vasopressin has been detected in rabbit plasma following injection into ligated colonic loops.[13] In addition, the bacterial chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) has been shown to be absorbed intact across rat colon.[14] In these experiments, dithiothreitol, which increases mucosal permeability, increased the uptake of fMLP in conjunction with an increase in ^{51}Cr-EDTA permeability. Apical to basal transepithelial absorption of intact horseradish peroxidase (HRP) across human colon carcinoma cell line (Caco-2) monolayers was not altered by low temperatures (4°C) or exposure to monensin, a drug known to inhibit receptor mediated endocytosis.[15] In contrast, basal to apical movement of intact HRP across Caco-2 monolayers is eight times greater than that observed in the apical to basal direction and is reduced at 4°C.

Macromolecular transport across the colonic mucosa may play an important role in normal intestinal function as well as pathophysiological states. The absorption of intact proteins may be

Abbreviations used in this paper: ATR, atropine; BSA, bovine serum albumin; HEX, hexamethonium; HRP, horseradish peroxidase; PD, potential difference; TTX, tetrodotoxin; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; TBS, Tris buffered saline; PBS, phosphate buffered saline.

involved in immune sampling and the development of tolerance.[16 17] Disturbances in macromolecular uptake may play a central role in disease states. Inflammatory diseases such as Crohn's disease have been shown to occur in conjunction with an increase in mucosal permeability,[18] which is thought to reflect an increase in luminal antigen absorption.[19] The large intestine is host to a variety of microorganisms[20] and the presence of normal intestinal flora has been linked to chronic colonic inflammation.[19] Bacterial byproducts such as the chemotactic peptide fMLP have been shown to cause an increase in intestinal permeability[21 22] and changes in intestinal permeability have been correlated with changes in intact protein transport.[5]

The present study was designed to characterise the kinetics and determine the mechanisms involved in the regulation of macromolecular antigen uptake in the proximal and distal large intestine.

Methods

ANIMAL MODEL

New Zealand white rabbits of both sexes weighing 1500–2000 g were obtained from a breeding colony (Vandermeer Inc., Edmonton, Alberta, Canada). Rabbits utilised in this study were maintained on a bovine serum albumin (BSA)-free diet. Animal care and experimental procedures followed the guidelines of the Canadian Council of Animal Care.

IN VITRO TRANSPORT STUDIES

Following an overnight fast rabbits were killed by an intravenous injection of euthanyl (0.5 ml/kg body weight). Immediately thereafter a segment of proximal or distal colon was removed and flushed with ice cold Krebs solution containing 115 mM NaCl, 8 mM KCl, 1.25 mM $CaCl_2$, 1.2 mM $MgCl_2$, 2 mM KH_2PO_4, and 25 mM $NaHCO_3$. Colonic tissue was placed over a glass rod and stripped of the muscularis. Tissues were then mounted in short circuited lucite Ussing chambers that exposed 1.9 cm^2 of tissue to 5 ml of Krebs solution. The mucosal and serosal solutions were circulated by a gas lift containing 95% O_2 and 5% CO_2 on each surface and the temperature was maintained at 37°C. The mucosal solution contained 10 mM mannitol (an inert sugar) to balance the serosal solutions osmotically which contained 10 mM glucose to provide metabolic energy to the tissue. The serosal solution also contained 50 μg/ml gelatin (Bio-rad, Richmond, California, USA) to prevent the binding of transported serosal BSA to charged surfaces of the Ussing chamber. Immediately after mounting the tissue was clamped at zero volts by an appropriate short circuit current (*I*sc) with an automatic voltage/current clamp apparatus (DVC-1000; World Precision Instruments), except for 5–10 seconds at 0, 50, 60, 70, and 80 minutes when the open circuit potential difference (PD) was measured. Tissue conductance (G, mS/cm^2) was calculated from PD (mV) and *I*sc (μA/cm^2) according to Ohm's law.[23] Fluxes of both immunologically intact, as measured by enzyme linked immunosorbent assay (ELISA), and BSA, determined by transport of ^{125}I-BSA, were assessed over three consecutive 10 minute periods following a 50 minute equilibration period. Samples (200 μl) were obtained from the serosal reservoir immediately after tissue mounting and at 50, 60, 70, and 80 minutes, and from the mucosal chamber at the start and end of the experiment.

TISSUE VIABILITY

During each experiment tissue viability was assessed by monitoring tissue electrical parameters. In addition, serosal and mucosal samples were obtained from proximal and distal colonic tissue over the course of the experiment and assayed for DNA content[24] as an indicator of tissue damage. At the end of four experiments on proximal colonic tissue and four experiments examining distal colonic tissue, chambered tissue was fixed in Carnoy's, sectioned, stained with haematoxylin and eosin, and histological assessment performed in a blinded manner. Sections were examined for evidence of epithelial disruption and oedema.

TRANSPORT KINETICS

To assess the kinetics of intact BSA (fraction V; Sigma, St Louis, Missouri, USA) transport, the mucosal surface was exposed to varying concentrations of BSA, ranging from 0.25 to 6.0 mg/ml. From the kinetic data obtained, intact macromolecular transport in the proximal colon was found to be maximal at a mucosal concentration of 4.0 mg/ml BSA. All subsequent experiments in proximal colonic tissue were performed at this concentration of BSA. Transport in distal colonic tissue reached a maximum at 1.0 mg/ml BSA. Due to the small magnitude of the movement of intact BSA in the distal colon, it was not feasible to assess the regulatory mechanism by pharmacological inhibition. In order to assess the total ^{125}I-BSA flux, 10 μCi ^{125}I-BSA (ICN, Mississauga, Ontario, Canada) was added to the mucosal surface in addition to 4.0 mg/ml BSA. Samples obtained for the measurement of immunologically intact BSA were maintained at 4°C for a maximum of 24 hours until assayed by ELISA for BSA content. Samples for measurement of total mucosal to serosal BSA movement (immunologically intact plus degraded BSA) were assayed for ^{125}I by scintillation spectrometry. Fluxes for both ELISA and ^{125}I-BSA experiments were calculated for individual 10 minute periods and an overall 30 minute flux as previously reported,[2] and are expressed as ng/cm^2/h.

REGULATION OF MACROMOLECULAR TRANSPORT

To examine the energy dependency of macromolecular uptake in the proximal colon and the role of microtubules in this process, tissue was exposed to 2×10^{-3} M sodium fluoride or 5×10^{-5} M colchicine (Sigma, St Louis, Missouri, USA) on both serosal and mucosal surfaces at time 0 and for the duration of the Ussing chamber experiment. Neuropharmacological blockade was achieved by the addition of 1×10^{-6} M tetrodotoxin (TTX; Sigma) to the sero-

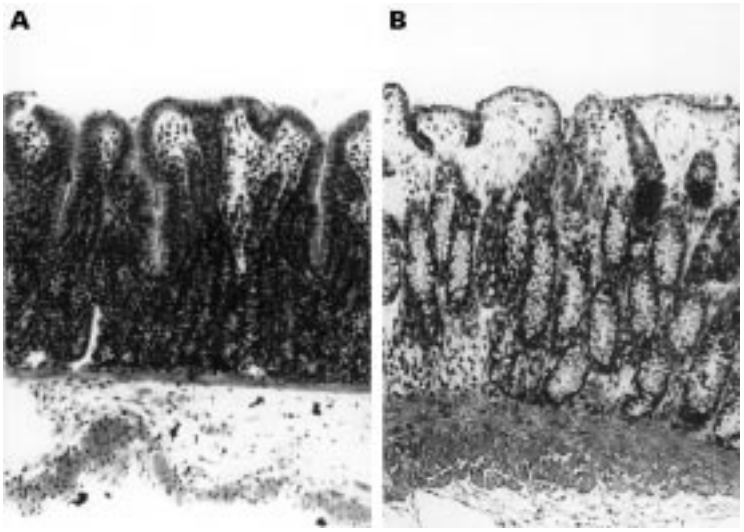

Figure 1 Representative micrographs of stripped colonic tissue obtained 80 minutes after mounting in the Ussing chamber. (A) proximal colon; (B) distal colon. Original magnification × 400.

sal bathing fluids at time 0. This dose of TTX was previously found to produce effective blockade. Complete neural blockade by TTX was confirmed by transmural electrical field stimulation at the end of the experimental period.[25] Cholinergic blockade was accomplished by the concurrent addition of atropine (ATR, 1×10^{-4} M) and hexamethonium (HEX, 1×10^{-4} M) to the serosal chamber at time 0.

The regulation of total ^{125}I-BSA flux in the proximal colon was assessed in separate experiments by the addition of sodium fluoride, colchicine, and TTX to the bathing fluids. The regulation of total BSA flux in the distal colon was assessed by the addition of sodium fluoride to the bathing fluid. All experiments using inhibitors and channel blockers were performed on paired tissues obtained from the same animal.

IMMUNOBLOTTING AND AUTORADIOGRAPHY

To determine whether the immunologically active BSA as measured by ELISA was partially degraded after transport across the mucosa, serosal samples were collected at 80 minutes and an immunoblot performed. Serosal solutions were concentrated using Ultrafree 20 protein purification filter tubes (Millipore, Nepean, Ontario, Canada) to ensure adequate visualisation of the transported BSA. Following purification samples were run on 10% non-denaturing linear gradient polyacrylamide gels.[26 27] Molecular weight markers (Sigma) ranging from 14.2 kDa to 132 kDa, freshly prepared BSA, and experimental samples were run concurrently. Gels were electrophoretically transferred onto nitrocellulose paper (0.4 μm pore size) using a Bio-Rad transblotting apparatus. The transblot was performed at 4°C while applying a constant current of 25 mA for 16 hours followed by an additional two hours at 200 mA. For immunoblotting, nitrocellulose sheets were blocked with 10% egg albumin (Fisher, Edmonton, Alberta, Canada) in Tris buffered saline (TBS) containing 0.05% Tween 20 (Sigma) for two hours. The transblot

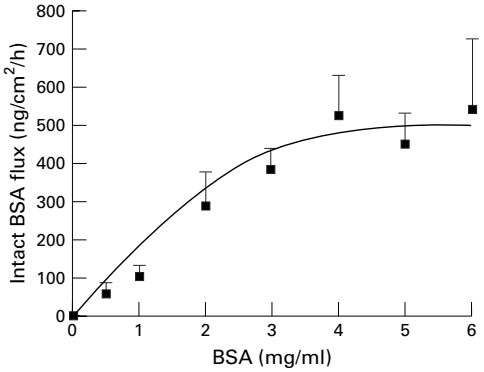

Figure 2 Dose response curve for intact BSA transport in the proximal colon. Data represent the mean (SEM) of at least three tissues at each concentration.

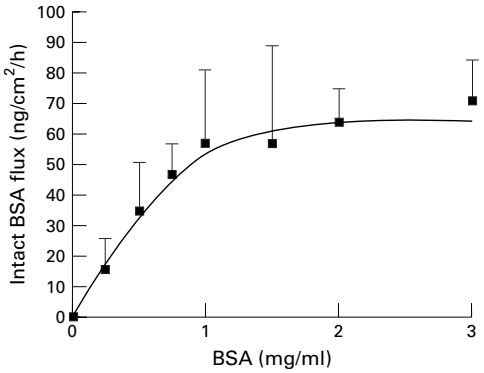

Figure 3 Dose response curve for intact BSA transport in the distal colon. Data represent the mean (SEM) of at least three tissues at each concentration.

was then incubated with rabbit anti-BSA HRP conjugate (Cappel, Scarborough, Ontario, Canada) diluted 1/2500 in TBS/Tween for 1.5 hours. Following three 15 minute washes in TBS/Tween and two 10 minute washes in TBS, the HRP colour reaction was performed using HRP colour development reagent (Bio-Rad, Richmond, California, USA).

To ensure that the ^{125}I radiolabel used to assess total BSA movement remained coupled to BSA prior to transport and during the experiment, mucosal samples were collected at the conclusion of the experiment. Samples were separated on 10% non-denaturing linear gradient polyacrylamide gels concurrently with molecular weight markers and freshly prepared ^{125}I-BSA. The gel was autoradiographed for 70 minutes with Kodak X-OMAT film to visualise the ^{125}I.

BSA ELISA

A two step sandwich ELISA was used to assay serosal samples for immunologically intact BSA as previously described.[2] Briefly, 96 well ELISA plates (Immulon 2; Dynatech, Chantilly, Virginia, USA) were coated with rabbit anti-BSA IgG (Oregon Technica, Scarborough, Ontario, Canada) diluted 1/500 in phosphate buffered saline (PBS) containing 0.05% Tween 20 and blocked with 10% egg albumin in PBS/Tween. Undiluted serosal samples as well as standards comprised of BSA of known concentrations (5, 10, 25, 50, 100, 250, 500, and 1000 ng/ml) were added and incubated for

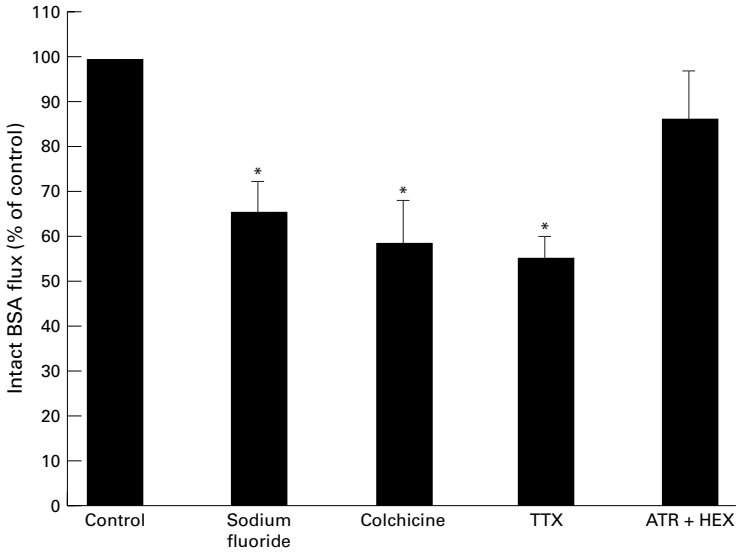

*Figure 4 Metabolic inhibition and neural regulation of intact BSA transport in proximal colonic tissue. NaF (n=6), colchicine (n=10), TTX (n=9), ATR + HEX (n=4), controls (n=the number in each experimental group). All measurements were performed in paired tissue. *p<0.05.*

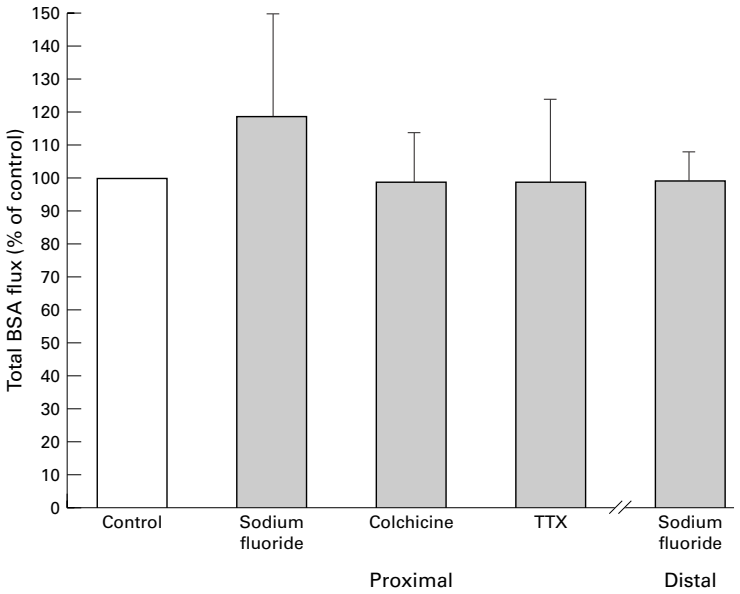

Figure 5 Metabolic, microtubule, and neural inhibition of total (intact and degraded) BSA. n=4 for all groups. All measurements were performed in paired tissue.

1.5 hours. After washing with PBS/Tween, rabbit anti-BSA IgG HRP conjugate diluted 1/500 in PBS/Tween was added and incubated for 1.5 hours. Following a final wash in PBS/Tween, the peroxidase colour reaction substrate was added. Optical density of the wells was read, and BSA concentration in samples determined from that of the BSA standards. Ouchterlony immunoprecipitations were performed by incubating rabbit serum versus serially diluted anti-BSA antibody in 10% agarose gel and staining for the precipitation bands. These results confirmed that there was no detectable cross reactivity to any rabbit tissue or serum proteins. The lower limit of assay sensitivity was 5 ng/ml. All samples giving optical densities of less than the 10 ng/ml standard are reported as having 0 ng/ml BSA.

KINETIC ANALYSIS

The kinetic data obtained from intact BSA flux experiments were analysed in order to determine whether immunologically intact BSA transport is a saturable process and to determine the magnitude of BSA transport. Analysis was performed by a non-weighted non-linear regression method as previously described.[7]

STATISTICS

Results were analysed by a Student's *t* test or where applicable by a paired Student's *t* test. Significance levels were set at $p<0.05$. All results are expressed as mean (SEM).

Results

TISSUE VIABILITY

After mounting, tissue *I*sc and PD were high, but rapidly achieved a stable baseline for the duration of the experiment. Tissue conductance was stable throughout all the experiments reported. Tissue *I*sc, PD, and G values over the course of the experimental period averaged 26 (2) $\mu A/cm^2$, 2 (0.2) mV, and 15 (1) mS/cm^2 in proximal colonic tissue, respectively, and 39 (4) $\mu A/cm^2$, 1.1 (0.1) mV, and 8 (1) mS/cm^2 in distal colonic tissue. DNA loss into the bathing solutions was small and did not increase significantly over the course of the experiment. For the proximal colon, at 0, 30, 60, and 90 minutes the mucosal solution contained 0.02 (0.004)%, 0.03 (0.004)%, 0.03 (0.004)%, and 0.03 (0.005)% DNA, and the serosal solution contained 0.02 (0.004)%, 0.03 (0.004)%, 0.03 (0.003)%, and 0.03 (0.005)% DNA, expressed as a percentage of total tissue DNA. Similar results were obtained when DNA loss was measured in chambered distal colonic tissue (data not shown). After 80 minutes in the Ussing chamber both proximal (fig 1A) and distal (fig 1B) colonic tissue appeared to be histologically normal. The epithelium was intact and there was no evidence of oedema. These data confirmed that our tissue preparations remained viable over the course of the experiments.

IN VITRO TRANSPORT

Figure 2 shows immunologically intact BSA uptake in proximal colon in the presence of varying BSA concentrations. Maximal intact BSA transport across proximal colonic tissue was achieved at a mucosal BSA concentration of 4.0 mg/ml. As shown in fig 3 the magnitude of the intact BSA flux across the distal colon was much smaller than that seen in the proximal colon and transport was maximal at a mucosal BSA concentration of 1 mg/ml. The dose response data obtained from intact BSA transport experiments were analysed by non-linear regression. Kinetic analysis showed that the proximal colon transported intact BSA with a V_{max} of 1021 (655) $ng/cm^2/h$ and a K_m of 6 (6) mg/ml while the distal colon transported intact BSA with a V_{max} of 96 (28) $ng/cm^2/h$ and a K_m of 0.99 (0.74) mg/ml. There was a consistent linear accumulation of BSA in the serosal compartment in all experiments over the initial 50 minute equilibration period and in the three subsequent flux periods. Three

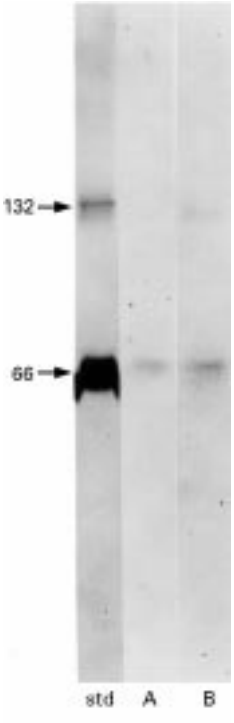

Figure 6 Immunoblot of BSA collected (A) fresh (immediately after preparation), and (B) from the serosal chamber after 80 minutes. Immunologically intact BSA is visualised as a single 66.2 kDa band in the sample obtained from (B).

consecutive 10 minute flux periods were calculated from the ELISA results and permitted a quantitative comparison between periods. There was no significant difference between the first, second, and third flux period in the proximal colon with a mucosal BSA concentration of 4 mg/ml (50–60 minutes, 596 (31) ng/cm^2/h; 60–70 minutes, 614 (30) ng/cm^2/h; 70–80 minutes, 732 (48) ng/cm^2/h) or 1 mg/ml BSA in the distal colon (50–60 minutes, 92 (48) ng/cm^2/h; 60–70 minutes, 50 (30) ng/cm^2/h; 70–80 minutes, 92 (47) ng/cm^2/h) indicating that intact BSA transport had achieved steady state conditions during the experimental periods. The mean BSA flux in the proximal colon of 665 (82) ng/cm^2/h was significantly greater ($p<0.01$) than that seen in the distal colon (78 (29) ng/cm^2/h). Intact BSA crossed the proximal colon as 7% of the total BSA flux. Intact BSA transport crossed the distal colon as 1% of the total BSA flux.

Total BSA movement across the proximal and distal colonic mucosa was measured by ^{125}I-BSA. The proximal segment showed steady state conditions over the three 10 minute periods (50–60 minutes, 9280 (2673); 60–70 minutes, 9731 (2303); 70–80 minutes, 10 951 (2949) ng/cm^2/h). The average flux for the three periods was 9938 (2252) ng/cm^2/h. The distal colon also displayed steady state conditions (50–60 minutes, 8185 (1175); 60–70 minutes, 12 025 (4236); 70–80 minutes, 8685 (1607) ng/cm^2/h) with an average flux of 8685 (1152) ng/cm^2/h.

REGULATION OF MACROMOLECULAR TRANSPORT

To elucidate the cellular mechanisms involved in the transport of intact BSA, BSA fluxes were measured following pharmacological and metabolic inhibition (fig 4). Sodium fluoride, which inhibits endocytosis by lowering cellular ATP levels,[28] significantly reduced transport of intact BSA by 36%. Colchicine, which depolymerises microtubules and impairs the movement of endocytosed material from apical to basal membranes,[29] also significantly reduced the passage of intact BSA into the serosal compartment by 42%. Macromolecular transport was also examined in the presence of a general neural antagonist. Tetrodotoxin (a Na^+ channel blocker) caused a significant 45% decrease in the uptake of intact BSA. Cholinergic blockade with atropine and hexamethonium did not significantly alter the uptake of intact BSA (fig 4). Tissue permeability was not affected by pharmacological manipulation. Conductance was not altered in the presence of sodium fluoride (12 (2) mS/cm^2), colchicine (13 (1) mS/cm^2), tetrodotoxin (15 (2) mS/cm^2), or atropine and hexamethonium (14 (2) mS/cm^2) compared with control values (12 (1) mS/cm^2). Figure 5 shows the effect of pharmacological inhibition on total BSA transport. Depletion of cellular ATP with sodium fluoride did not significantly reduce total BSA flux in either proximal or distal colon. Furthermore, addition of colchicine and tetrodotoxin had no effect on total BSA transport in the proximal colon (fig 5).

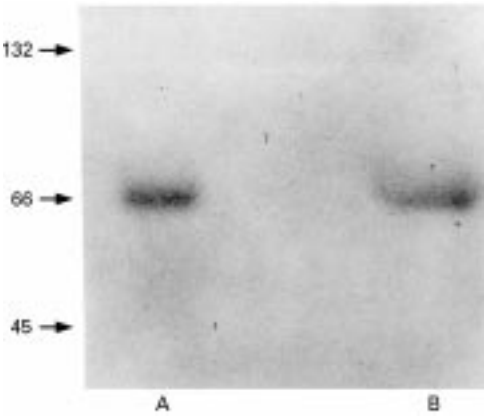

Figure 7 Autoradiograph of mucosal solution obtained at 80 minutes containing ^{125}I-BSA (A) and freshly prepared ^{125}I-BSA (B). BSA was not degraded prior to transport across the tissue and remained conjugated to ^{125}I.

IMMUNOBLOTTING AND AUTORADIOGRAPHY

Serosal samples collected from proximal colonic tissue experiments at 80 minutes showed that after transport, the immunologically active material represented a single band of similar molecular weight to that of freshly prepared BSA. No immunologically active fragments were seen (fig 6).

Autoradiography of mucosal samples that were collected at the end of the experiment indicated that all radioactivity in the sample was contained in a band of similar molecular weight to that of the freshly prepared ^{125}I-BSA and the BSA standard. No free ^{125}I was seen at the ion front or in association with smaller molecular weight proteins (fig 7).

Discussion

These studies show that the large intestine is capable of sampling luminal macromolecules and that this process is energy dependent, requires microtubules, and is neurally regulated.

The stability and viability of colonic tissue preparations were carefully assessed: histological evaluation of stripped tissue showed a consistent, clean separation of the external muscle coats from the submucosa and there was no evidence of epithelial or mucosal disruption of the tissue during the 80 minute experimental period. Tissue electrical parameters stabilised rapidly following mounting of the tissue. By 50 minutes, when macromolecular sampling began, tissue PD, *I*sc, and G had achieved a stable baseline and remained stable over the course of the experiment. To confirm further the viability of our colonic tissue preparations we measured DNA loss into the chamber bathing fluid. DNA loss was very low and did not change appreciably during the experiment. Furthermore, macromolecular flux was in steady state conditions, in both the proximal and distal segments, between 50 and 80 minutes.

In the current study, intact BSA transport in the proximal colon was approximately five times greater than intact BSA transport in the distal segment. The values for BSA transport in the proximal colon (total flux 9938 (2252)

ng/cm^2/h; intact flux 665 (82) ng/cm^2/h) are similar in magnitude to that reported for BSA uptake in the small intestine. Values for total BSA flux in rat jejunum ranged from 11 860 (1917)[6] to 13 874 (961) ng/cm^2/h[7] with an intact component measuring from 356 (59) to 665 (32) ng/cm^2/h respectively. Other studies examining HRP transport have reported rates of total HRP transport ranging from 2410 (397) ng/cm^2/h (intact 352 (75)) in mouse jejunum[16] to 4004 (1408) ng/cm^2/h (intact 137 (26)) in rabbit jejunum.[4] Human infant jejunum transports HRP with a total flux of 1734 (306) and an intact flux of 260 (55) ng/cm^2/h.[30] β Lactoglobulin is transported across rabbit ileum with a total mucosal to serosal flux of 5460 (1750) ng/cm^2/h and an intact component of 430 (80) ng/cm^2/h.[9] In Caco-2 cells, total apical to basal HRP flux occurs at a rate of 1206 (392) ng/cm^2/h with an intact flux of 392 (154) ng/cm^2/h.[15]

Intact BSA transport as a percentage of total BSA transport was 7% in the proximal colon compared with 1% in the distal colon. Reports on the percentage of total HRP being transported intact across rabbit and mouse jejunum vary from 3%[4] to 15%.[16] In human infant jejunum 12% of the total HRP flux crosses the epithelium intact.[30] In rabbit ileum the intact protein flux represents 6–9% of the total β lactoglobulin flux.[9] In rat jejunum 3–4.5% of BSA crosses the epithelium intact.[6 7] Similarly, intact HRP flux crosses Caco-2 monolayers as 3% of the total HRP flux.[15] In rat stomach intact BSA transport comprises 27% of the total BSA flux[2] though the magnitude of the total flux is much lower than that reported for small and large intestinal tissue.

Experiments were performed to define the mechanisms involved in the absorption of macromolecules across the colonic epithelium. Intact macromolecular transport in the proximal colon occurred via an energy dependent transcellular process that utilised the microtubular network and was regulated by the enteric nervous system. In the distal colon the intact flux was too small to assess pharmacological inhibition accurately. The findings in terms of energy dependency, the need for microtubules, and neural regulation are similar to those defined for other regions of the gastrointestinal tract. Intact macromolecular uptake has previously been shown to be energy dependent in rat,[7 31] rabbit,[4 9] and piglet[32] small intestine and rat stomach.[2] In contrast, mucosal to serosal transport of intact HRP was not altered by metabolic inhibition in Caco-2 monolayers though serosal to mucosal transport was.[15] These findings emphasise the potential pitfalls associated with transformed cell lines and suggest caution in extrapolating findings obtained in transformed cell systems to the intact organism. Transcytosis of intact proteins has also been shown to require microtubules in rat and rabbit small bowel[4 7 9] and rat stomach.[2] We have previously shown a role for the enteric nervous system in the regulation of intact jejunal macromolecular uptake[7] and the cholinergic agonist carbachol has been shown to upregulate intact HRP uptake in rat ileum.[10] The data from the current study show that intact macromolecular transport in the proximal colon is also neurally regulated though this regulatory influence does not appear to be exerted via cholinergic pathways.

In contrast to the findings for intact macromolecular uptake, the movement of degraded protein does not appear to be regulated in colonic tissue. Metabolic inhibition, microtubule depolymerisation, and neural blockade had no effect on the total flux of BSA across the proximal colonic epithelium despite the effect of these treatments on intact macromolecular transport in the proximal colon. Likewise, metabolic inhibition did not significantly alter the total flux of BSA across the distal colon. However, the intact flux of protein represents such a small component of the total macromolecular flux that inhibition of the intact flux would not necessarily be reflected by alterations in the total flux. We have previously reported a decrease in total macromolecular flux following metabolic inhibition in rat jejunum[7] and rat stomach[2] and total mucosal to serosal HRP flux in Caco-2 monolayers and β lactoglobulin transport in rabbit ileum have also been shown to be energy dependent.[9 15] The reason for this discrepancy is not known though it may be related to the increased metabolic activity present in gastric and small intestinal tissue compared with the colon. Total β lactoglobulin flux was also reduced following microtubule depolymerisation in rabbit ileal tissue.[9]

A transblot performed on solutions obtained from the serosal compartment at the end of the experiment revealed that transported intact BSA represented a single band of similar molecular weight as that of freshly prepared BSA indicating that BSA is unaltered when moved via the intact pathway. Autoradiography performed on the ^{125}I-BSA solutions in the mucosal compartment indicated that all the ^{125}I remained conjugated to intact BSA prior to transport across the tissue. Therefore, degradation of BSA, production of ^{125}I labelled protein fragments, and dissociation of label from the protein must be occurring subsequent to uptake across the apical surface of the epithelium and during transport across the tissue.

A model for the transcellular, pinocytotic uptake and transport of intact macromolecules has been proposed. Proteins are thought to bind to receptors on the surface of the brush border membrane which then invaginates to form phagosomes containing the protein. Phagosomes are transported via microtubules to lysosomes where most fuse to form phagolysosomes with subsequent digestion of their contents. The contents of the phagolysosomes, along with phagosomes that have failed to fuse, is then delivered across the basolateral membrane by exocytosis.[33] Each of these steps is energy dependent. This model is supported by experiments showing an increase in intact small intestinal macromolecular flux following treatment with ammonia, an agent which inhibits lysosomal proteolytic activity.[4 9] Conversely, recent experiments suggest that under certain circumstances (antigen challenge in

sensitised animals), a significant amount of intact protein may cross the epithelial barrier via a paracellular route.[34]

As discussed above, the magnitude of the flux of intact protein is much greater in the proximal colon compared with the distal segment. This likely reflects the fact that the proximal colon is exposed to a greater amount of protein, from both dietary and endogenous (bacterial) sources, than is the distal colon. Thus, the proximal colon is probably the primary site for macromolecular sampling in the large bowel. The physiological basis for the lower flux of intact protein in the distal colon is not known. Electrical conductance is decreased in the distal colon compared with the proximal colon due to a greatly reduced passive ion conductance. However, the data from the current study suggest macromolecular transport in the colon is an active, transcellular process. It remains to be determined whether alterations in the level of lysosomal activity may play a role in regulating the magnitude of the flux of intact protein in different segments of the gut.

Macromolecular sampling by the colon may play an important role in both normal physiology as well as pathophysiological states. Enterocytes can express MHC II and may function as antigen presenting cells.[35] Thus the absorption of intact macromolecules by the colonic mucosa may be involved in immune sampling and the development of tolerance.[16 17] Macromolecular sampling may also provide a mechanism for luminal surveillance, allowing the body to monitor colonic bacterial loads or assess small intestinal digestive function. Furthermore, alterations in macromolecular uptake may play a central role in the aetiology of intestinal inflammatory disease states. Patients with Crohn's disease and their relatives display an increased intestinal permeability to inert markers[18] suggesting an underlying primary defect in barrier function as a precondition for disease onset. Patients with Crohn's disease also display increased intestinal permeability to macromolecules[36] and work in experimental models of colitis suggests the presence of normal colonic flora plays an important pathogenic role in the development of chronic lesions.[19] Moreover, a variety of pathological conditions have been shown to alter intestinal macromolecular permeability. Malnutrition,[35] rotavirus infection,[37] and bacterial infections such as enteroadherent *Escherichia coli*[38] have all been shown to result in an increase in epithelial macromolecular permeability. In addition, macromolecular transport plays a prominent role in intestinal food allergies by providing a pathway for the uptake and presentation of the sensitising antigen to mucosal mast cells and subsequent triggering of the anaphylactic response. Allergic colitis to food protein is a recognised entity in infants.[39]

Accumulating evidence suggests that macromolecular sampling is regulated by the enteric nervous system. The enteric nervous system has been proposed to play a role in regulating intestinal immune function[40] and there is a close anatomical relation between elements of the enteric nervous system and the intestinal immune system. Mucosal mast cells, a prominent immune effector cell type, have been shown to be in close contact with elements of the enteric nervous system.[41–43] Furthermore, nerve mediated antigen uptake in rat jejunum is enhanced in the presence of bacterial (*Bordetella pertussis*) toxin.[44]

In summary, the findings indicate that the large intestine actively transports antigenically intact protein and that the majority of transport occurs in the proximal segment. Colonic macromolecular BSA transport is a saturable, microtubule and energy dependent transcellular process that is regulated by the enteric nervous system.

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