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## A simplified procedure for studies of intestinal immunity in rabbits

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### Abstract

As interest in the development of oral vaccines continues to rise, alternative animal models for studies of mucosal immunity are needed. The present study examines a simplified procedure for delivering antigen to rabbit Peyer's patches via an indwelling cannula. The cannula was placed 3–4 cm proximal to the Peyer's patch, and was used to deliver four weekly doses of the potent mucosal immunogen, cholera toxin (CT). Anti-CT specific fecal secretory IgA (S-IgA), serum IgG and serum IgA were found in essentially equal amounts in rabbits with cannulas and in rabbits fitted with Thiry-Vella (T-V) isolated ileal loops. In contrast to animals with T-V loops, the intestinal flora of animals with cannulas contained less bacterial overgrowth with *Pseudomonas* sp. Further, the villus architecture remained histologically normal in appearance and there were fewer post-surgical complications associated with this technique than with T-V loops. This simplified technique should allow wider use of rabbits in studies of mucosal immunity.

**Keywords:** Mucosal immunology; Secretory IgA; (Rabbit)

### 1. Introduction

Most pathogenic organisms must either colonize, attach, invade, or traverse a mucosal surface before establishing infection in a host organism. A multitude of vaccine candidates have been tested for the ability to elicit mucosal immunity to pathogenic organisms and their toxins. However, only a limited number of well characterized animal models are available for such studies, with most employing inbred mice. The rabbit model has been used exten-

sively for studies of hepatobiliary transport of S-IgA (Kuhn and Kraehenbuhl, 1979a,b,1981), and as a model for both the pathogenesis and immune response to numerous human enteric diseases. These include rotavirus (Conner et al., 1991), *Vibrio cholerae* (Kabir, 1993; Majumdar and Ghose, 1981, 1982; Rijpkema et al., 1992), *Salmonella* (Keren et al., 1982), *Shigella* (Keren et al., 1989a), and *E. coli* (Fasano et al., 1991). The exceedingly slow gastric motility of the rabbit can compromise the stability of orally administered protein antigens, making direct injection of vaccine preparations to the intestinal mucosa more desirable.

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Chronically isolated intestinal Thiry-Vella (T-V) loops have been used to study mucosal immunity in rabbits (Keren et al., 1980b, 1989a,b; Wassef et al., 1989; Silbart et al., 1992; Kern et al., 1987) and other species (Husband and Lascelles, 1974; Porter et al., 1974; Sack et al., 1976; Mebus et al., 1976). This model has proven valuable as both a direct route for mucosal immunization as well as an access port for collecting intestinal secretions after local stimulation. There are several inherent shortcomings to surgical isolation of the intestine which reduce the utility of the T-V loop model. Chief among these are morphological changes in the villus epithelium resulting from isolation of the loop from chyme (Keren et al., 1975), and chronic mucosal inflammation due to bacterial overgrowth in the loops (Keren et al., 1980a, Kern et al., 1987). In addition, the technique is time consuming, technically difficult, and can result in post-surgical complications such as localized peritonitis, intestinal volvulus, and surgical adhesions which can occlude intestinal blood flow. In the present study a simplified method for delivering antigenic material to rabbit Peyer's patches was employed using a procedure similar to that described by Tadano et al. (1992) for rats and by Meunier et al. (1993) for dogs. Intestinal cannulation was quite

effective for delivery of antigen to Peyer's patches, yet suffered few of the shortcomings of T-V loops.

## 2. Materials and methods

### 2.1. Preparation of isolated (T-V) ileal loops

Chronically isolated ileal (Thiry-Vella) loops were prepared as described by Keren et al. (1975). Female New Zealand White specific pathogen free rabbits weighing 2–3 kg were fasted for 12–18 h prior to surgery. Each was anesthetized with 100 mg/kg of ketamine HCl (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) and 20 mg/kg xylazine (Anased, Lloyd Laboratories, Shenandoah, IA) i.m., followed by a mixture of 50 mg/kg ketamine HCl and 10 mg/kg xylazine i.m. as needed (about every 20–30 min). Following a midline incision, 15 cm of ileum containing the most distal Peyer's patch was surgically isolated by transecting the intestine proximal and distal to the segment. While the mesentery was cut to permit movement of the excised segment, care was taken to insure that the blood supply to the segment was left intact. Continuity of the intestine was restored by end-to-end anastomosis using con-

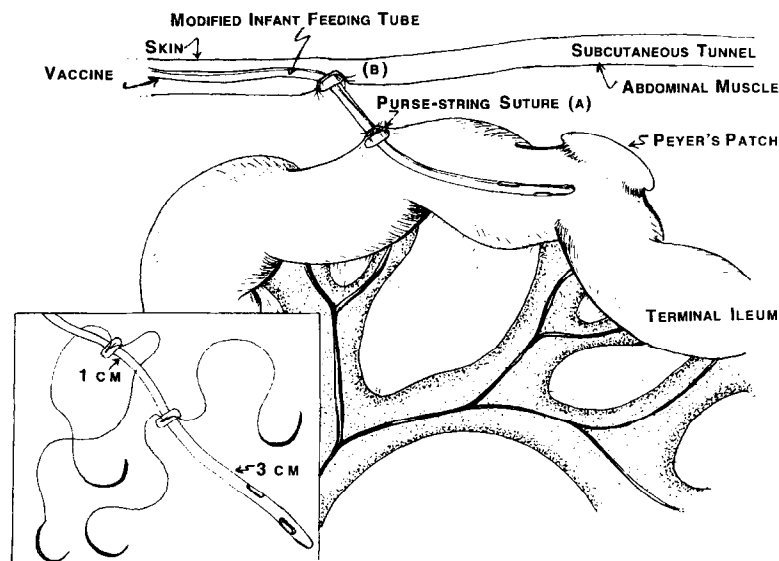


Fig. 1. Placement of intestinal cannula.

tinuous 4-0 silk sutures. Two tubes (Silastic type, Dow Corning, Midland, MI) were sewn into the ends of the isolated segment using a purse string suture of 4-0 silk (Deknatel, Queens Village, NY) and then anchored to the body wall with 3-0 silk (Deknatel) upon closing. Both access tubes were brought through a subcutaneous tunnel and exteriorized at the nape of the neck. A plastic, Elizabethan-style collar was used to prevent the rabbit from damaging the tubes.

### 2.2. Cannulation of the ileum

Cannulas were prepared by modifying infant feeding tubes (8Fr 15", Baxter Healthcare, Deerfield, IL) with circumferential beads of Silastic medical adhesive (Dow Corning) with 4-0 silk suture material (with swaged 12 mm cutting needles attached) embedded in the adhesive prior to curing (Fig. 1). Each rabbit was anesthetized and prepared for surgery as describe above. Following a midline incision, the distal ileum was located and perforated 3–4 cm proximal to the most distal Peyer's patch, on the anti-mesenteric side, with an 18 gauge needle. The cannula was introduced through the hole with the vented end directed distally within the lumen of the ileum. The perforation was closed around the cannula with a single purse string suture using the attached 4-0 silk (labeled A in Fig. 1). A second piece of 4-0 silk (labeled B in Fig. 1) was used to anchor the cannula to the body wall at the anterior end of the incision, leaving the intestine within the body cavity. The free end of the cannula was then tunneled subcutaneously, exteriorized near the nape of the neck, and protected with a plastic collar as described above. The skin was closed separately, and a final suture was made where the cannula was exteriorized. To prevent the escape of intestinal contents from the cannula, the exteriorized end was capped until vaccine preparations were introduced.

### 2.3. Immunizations

Rabbits with Thiry-Vella loops were immunized on days 2, 9, 16 and 23 after surgery with 0.1 mg of CT (azide free, List Biological Laboratories, Campbell, CA) in 4.0 ml of PBS (0.01 M  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  0.9% NaCl, pH 7.3). Rabbits with intestinal cannulas were immunized on the same

days with an identical dose of CT in 1 ml of PBS. The vaccine was cleared from the lumen of the cannula by injecting an additional 1 ml of PBS.

### 2.4. Sample collection

Rabbits were bled via the central ear vein prior to surgery and weekly thereafter. Blood samples were allowed to clot by overnight refrigeration, then centrifuged at  $1500 \times g$  for 5 min. The serum was removed and stored at  $-20^\circ\text{C}$  until assay. Ileal loop secretions were collected daily by injecting air into one access tube, which ejected approximately 1–2 ml of loop fluid from the opposing tube. The loops were then flushed with 10 ml of 0.9% sterile saline followed by 10 ml of air to clear the loop. The recovered secretions were then centrifuged at  $1500 \times g$  for 5 min to separate mucus and cell debris, and the supernatant was stored at  $-20^\circ\text{C}$  until assay.

### 2.5. Fecal antibodies

Fresh feces were collected over a 1 h period on a weekly basis, and coproantibodies isolated following the procedure described by DeVos and Dick (1991). Briefly, to a 1 g fecal sample were added 10 ml of PBS followed by a 15 min incubation at room temperature. The fecal mass was then vigorously vortexed until a homogeneous mixture was obtained, then centrifuged at  $1500 \times g$  for 15 min at room temperature. The supernatant was then removed and stored at  $-20^\circ\text{C}$  until assay.

### 2.6. ELISA

Microtiter plates (Immulon 4, Dynatech Laboratories, Chantilly, VA) were divided into sets of three columns and coated with CT in the first column, BSA (bovine serum albumin, Sigma Chemical Co., St. Louis, MO) in the second column and coating buffer alone (0.05 M  $\text{Na}_2\text{CO}_3$ , pH 9.6) in the third column. The plates were coated by adding 100  $\mu\text{l}$  of a 10  $\mu\text{g}/\text{ml}$  protein solution (in coating buffer) to each well, then tightly covering each plate with Parafilm laboratory film and incubating overnight at  $25^\circ\text{C}$ , then storing the plates at  $4^\circ\text{C}$  until use. Immediately prior to use, the plates were washed in PTA (0.05 M  $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ , 0.1% Tween 20, 0.02%

sodium azide, pH 7.4) and then 100  $\mu$ l of diluted serum, intestinal secretion or fecal supernatant samples were applied to the plates and incubated for 4 h at 4°C. The plates were then washed with PTA and the appropriate dilution of alkaline phosphatase conjugated secondary antibody was applied (either goat anti-rabbit IgG or goat anti-rabbit IgA) and incubated overnight at 4°C. After washing with PTA, alkaline phosphatase substrate was added (*p*-nitrophenyl phosphate (Sigma), 1 mg/ml in substrate buffer (0.05 M Na<sub>2</sub>CO<sub>3</sub>, 1.0 mM MgCl<sub>2</sub>, pH 9.8), and the absorbance measured at 405 nm after 25, 50 and 100 min incubations at 25°C. In order to normalize the data to correct for inter-plate variation, a positive control was included consisting of an identical aliquot of high-titer anti-CT antiserum (1/30 000 dilution) or intestinal secretions (1/50 dilution). The mean 100 min absorbance from two duplicate BSA wells was subtracted from that of two duplicate CT wells to give a net anti-CT response for each sample. This net absorbance was then normalized by multiplying it by the quotient of the plate positive control divided by the group mean of all positive control values obtained from all plates in the study.

### 2.7. Culture and identification of intestinal bacterial flora

Each experimental animal was killed by lethal injection of sodium pentobarbitol (50 mg/kg, i.v.) 32 days after the initial immunization. The ileum was exposed by a midline incision and a 5 cm segment was selected for culture as follows: For rabbits with cannulas, the ileum was clamped just proximal and distal to the site of cannulation, then 1 ml of anaerobic thioglycollate (TG) media (BBL enriched thioglycollate medium PR11, Becton Dickinson Microbiology Systems, Cockeysville, MD) was injected into the isolated segment. This segment was briefly hand-agitated, then 0.55 ml of fluid was removed with a syringe previously purged with anaerobic gas and immediately injected into an anaerobic culture tube containing 5 ml of TG media. For rabbits with T-V loops, 0.55 ml of the fluid which accumulated in the section (1–2 ml/24 h) was withdrawn, diluted and plated identically to those of the cannulated rabbits. All anaerobic culture

dilutions and plating were performed in an anaerobic chamber (AtmosBag, Aldrich Chemical Co., Milwaukee, WI) after a 5 min purge with oxygen free gas (80% N<sub>2</sub>, 10% CO<sub>2</sub>, 10% H<sub>2</sub>, Cryodyne Technologies, Chester, CT). Serial dilutions of 1/10 through 1/10<sup>7</sup> were prepared and 0.1 ml aliquots of each dilution were applied to both anaerobic and aerobic plates in parallel. The anaerobic plating was performed using tryptic soy agar (TSA) with 5% sheep blood (BBL prepared media, Becton Dickinson) for 48 h. The aerobic plating was performed using TSA plates (Difco Laboratories, Detroit, MI) for 24 h, both at 37°C. Anaerobic conditions were maintained using the Gaspack Anaerobic system (BBL Microbiology Systems, Becton Dickinson) and verified using Resazurin as an anaerobic indicator dye (Sigma Chemical Co.). Isolates were identified using the API 20E microtube system (BioMerieux Vittek, Hazelwood, MO). Bacterial density for each animal was determined by counting colonies and multiplying by the appropriate dilution factor.

### 2.8. Preparation of histological samples

After cultures were taken, the isolated loop or segment of ileum containing the cannula (10 cm proximal and distal to the point of cannula insertion) was excised. For untreated control rabbits a 20 cm segment of distal ileum containing a Peyer's patch was excised. The excised segments were then opened longitudinally along the mesenteric border, secured to a flat surface and fixed in neutral buffered 10% formalin. The samples were then processed in a graded series of alcohols and toluene, embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin.

## 3. Results

### 3.1. Mucosal and systemic immune response to CT

The serum and feces collected from cannulated or T-V loop animals were assayed by ELISA for anti-CT responses. The serum IgG responses were extremely strong (titer > 30 000) for both groups of rabbits, with the T-V loop rabbits demonstrating a slightly higher response than the cannulated rabbits (Fig.

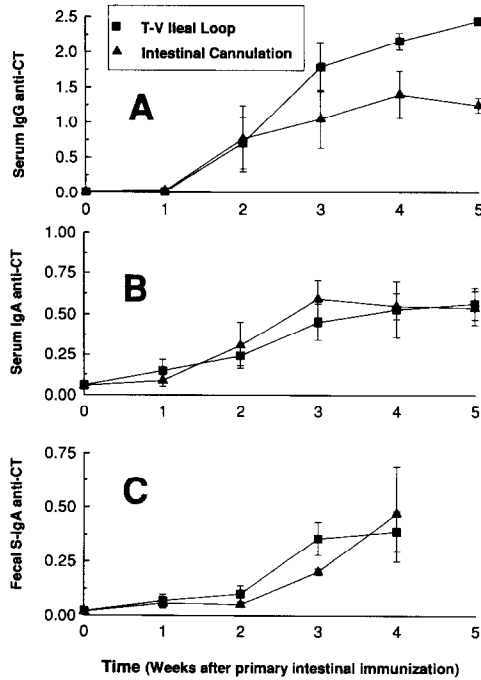


Fig. 2. Serum and fecal anti-CT immune response. A: serum samples were diluted 1/30000 and assayed by ELISA as described in Section 2. B: for serum IgA assays, each sample was diluted 1/50. C: fecal supernatant anti-CT S-IgA was assayed at a 1/10 dilution. Data are expressed as the mean optical density ( $\lambda = 405$  nm) measured 100 min after substrate addition  $\pm$  the standard error of the mean ( $n = 4$ ).

2A). Although the anti-CT serum and fecal IgA titers were substantially lower than the serum IgG (50 and 10 respectively), these responses were essentially

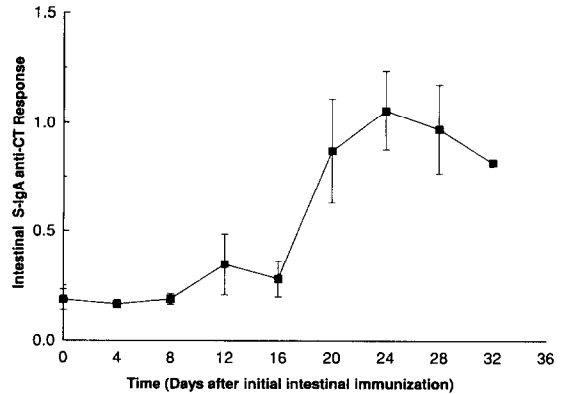


Fig. 3. Intestinal anti-CT S-IgA from Thiry-Vella ileal loop secretions. ELISAs were performed on samples diluted 1/50. Data are expressed as the mean optical density ( $\lambda = 405$  nm) measured 100 min after substrate addition  $\pm$  the standard error of the mean ( $n = 4$ ).

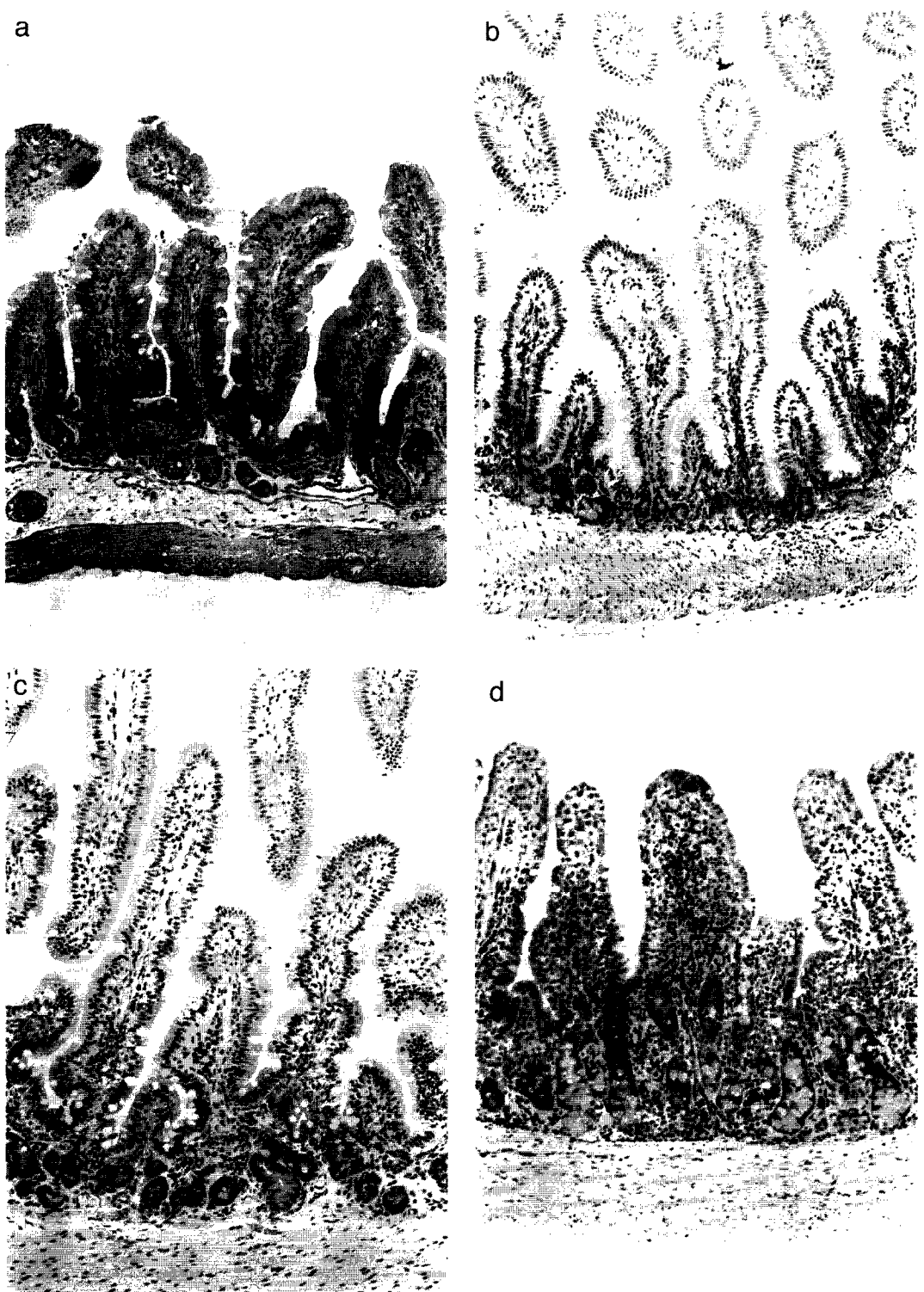
equal between the two treatment groups (Fig. 2B and Fig. 2C). Direct antigenic stimulation of the T-V loop over a 24 h time period resulted in significantly higher anti-CT S-IgA titers in ileal secretions than in the feces of either group (titer = 50, Fig. 3).

### 3.2. Intestinal microbial flora

*Pseudomonas aeruginosa* was the most prevalent bacterium colonizing the T-V loops ( $10^5$ – $10^6$  colony forming units (cfu)/ml of ileal contents), however *Staphylococcus* and *Bacteroides* were also found. Overall, T-V loops averaged  $2.9 \times 10^6$  cfu/ml for the anaerobic culture and  $2.7 \times 10^6$  cfu/ml for the

Table 1  
Intestinal microbial flora of rabbits with intestinal cannulas, T-V loops and controls

Treatment group	Animal no.	Anaerobe maj. sp.	no. of colonies	Aerobe maj. sp.	no. of colonies
Intestinal cannula	CT-2	<i>Bacteroides</i> sp.	$5.4 \times 10^5$	<i>E. coli</i>	$6.7 \times 10^4$
	CT-4	<i>Proteus mirabilis</i>	$9.2 \times 10^6$	<i>Proteus mirabilis</i>	$4.4 \times 10^6$
	CT-6	<i>E. coli</i>	$1.3 \times 10^5$	<i>E. coli</i>	$6.6 \times 10^4$
	CT-12	<i>Bacteroides</i> sp.	$1.1 \times 10^6$	<i>Enterobacter</i>	$1.7 \times 10^5$
Thiry-Vella loop	CT-5	<i>Staphylococcus</i> sp.	$2.0 \times 10^6$	<i>Pseudomonas aeruginosa</i>	$3.2 \times 10^5$
	CT-7	<i>Bacteroides</i> sp.	$3.8 \times 10^6$	<i>Pseudomonas aeruginosa</i>	$5.0 \times 10^6$
	CT-23	<i>Bacteroides</i> sp.	$8.0 \times 10^2$	<i>Pseudomonas aeruginosa</i>	N.D.
	CT-24	<i>Bacteroides</i> sp. and <i>Corynebacteria</i> sp.	$6.8 \times 10^5$	<i>Pseudomonas aeruginosa</i>	$7.0 \times 10^5$
Controls	CT-21	<i>Proteus mirabilis</i>	$1.0 \times 10^4$	<i>Pseudomonas aeruginosa</i>	$3.2 \times 10^4$
	CT-22	<i>Proteus mirabilis</i> and <i>E. coli</i>	$2.4 \times 10^5$	<i>Pseudomonas aeruginosa</i>	$2.8 \times 10^5$
	CT-25	<i>E. coli</i> and <i>Bacillus</i>	$1.6 \times 10^4$	<i>E. coli</i>	$1.6 \times 10^4$
	CT-26	<i>E. coli</i> and <i>Bacillus</i>	$3.2 \times 10^3$	<i>E. coli</i>	$1.4 \times 10^4$



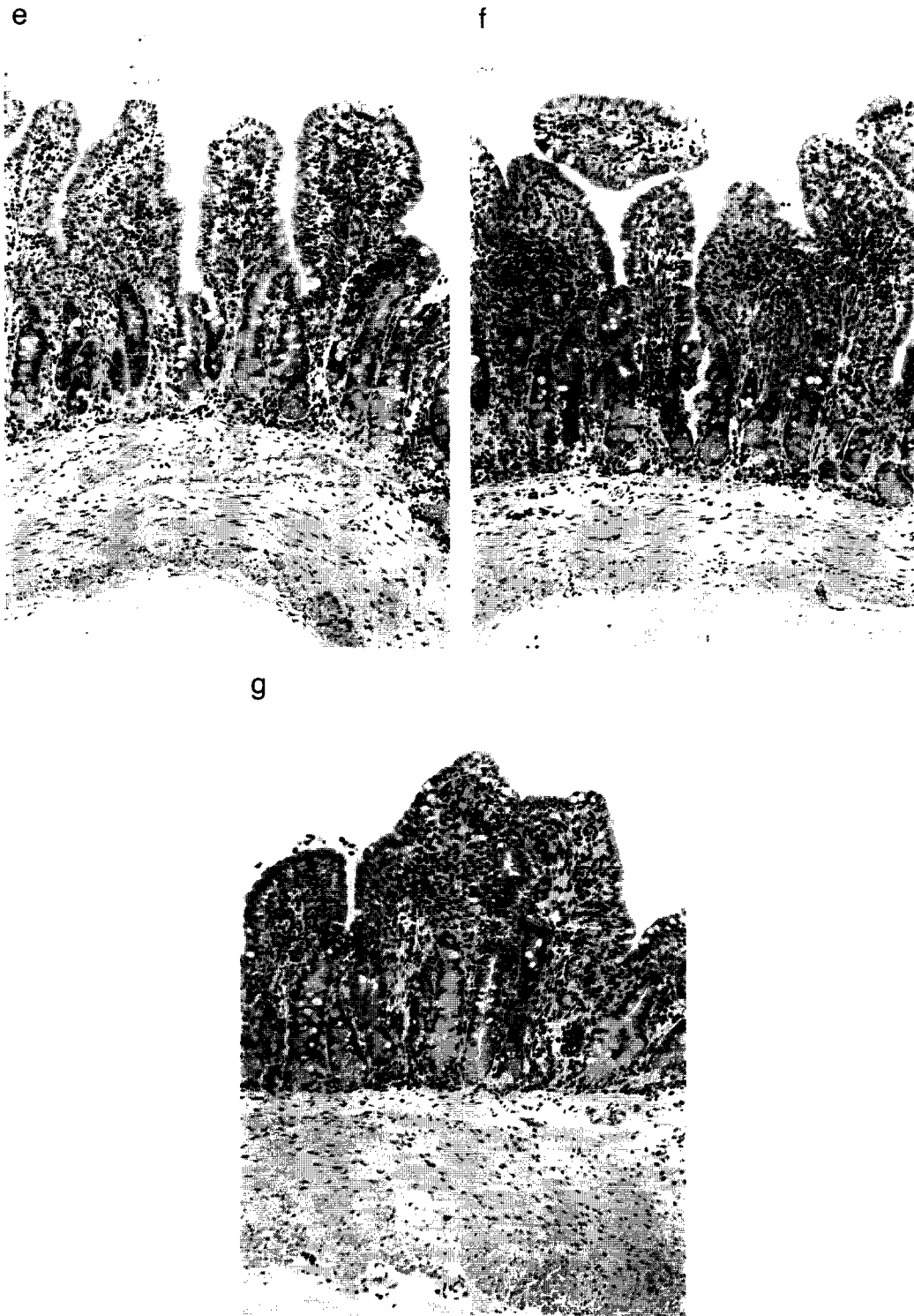


Fig. 4 (continued).



aerobic culture. No *Pseudomonas* species were isolated from rabbits fitted with ileal cannulas, however this organism was observed in smaller numbers in two of four untreated control rabbits (Table 1).

### 3.3. Histologic findings

The ilea of rabbits fitted with cannulas showed no pathologic changes (Fig. 4a–c), while the T-V loops were found to be shortened, with evidence of chronic inflammatory changes (Fig. 4d–g). Villi were broadened and shortened, with a loss of scalloping. The epithelium was cuboidal rather than columnar, and many cells had degenerative changes. The lamina propria of the villus cove was filled with lymphocytes and plasma cells.

## 4. Discussion

Direct administration of mucosal vaccines to rabbit ileum via an indwelling cannula provides a convenient model for studies of mucosal immunity. Several anatomical and physiological features of the rabbit intestine offer advantages over rodent models. First, the rabbit intestine contains numerous, easily distinguishable Peyer's patches, each of which contains a large numbers of lymphocytes. Secondly, the majority of S-IgA secreted into the rabbit intestine is locally produced by resident plasma cells in the lamina propria. This parallels human intestinal immunity, as the majority of serum IgA in both species is in monomeric form. Rodents have substantially more dimeric IgA in serum, which is efficiently transported via the polymeric immunoglobulin receptor (pIgR) expressed on epithelial cells lining liver sinusoids. Once bound to pIgR, the complex is transcytosed to the bile as S-IgA, and ultimately secreted into the intestinal lumen. Finally, direct administration of antigen to the intestine allows a more precise estimate of the dose delivered, and affords a higher

degree of confidence regarding antigen stability. Studies in rodents rely on the administration of antigen to the stomach, in large volumes of bicarbonate buffer, via orogastric feeding needles. The transit time to the intestine, degree of acidification, and percentage of intact antigen which reaches the intestinal inductive sites is generally unknown.

In the present study, a simple alternative to Thiry-Vella isolated intestinal loops was examined as a means for delivering antigen to rabbit Peyer's patches. The surgical insertion of an indwelling intestinal cannula was straightforward, requiring only about one half of the time of T-V loop surgery. Post-surgical complications were minimal, with only minor infection of the subcutaneous tunnel observed in an occasional animal. None of the post-surgical complications associated with T-V loop surgery were observed.

Following administration of four weekly doses of CT, the anti-CT serum IgG responses were slightly higher in animals with T-V loops than in animals with intestinal cannulas. This may have occurred due to the constant stimulation of the Peyer's patch in cannulated animals. Nonetheless, both groups of animal elicited high titer serum IgG anti-CT responses (> 30 000). The anti-CT serum IgA and fecal S-IgA responses were comparable in both groups of animals, indicating that both techniques were capable of stimulating B and T cells of the patch to mature, proliferate, traffic, and ultimately populate the effector tissues of the gut (viz. the lamina propria). For the purposes of comparison, only the most distal ileal Peyer's patches were used in both groups of animals. It seems likely that if the intestinal cannula was placed more proximal, perhaps even in the jejunum, that mucosal responses would be even higher due to stimulation of multiple patches.

Ileal loop secretions had higher anti-CT S-IgA responses than the fecal samples from either group of animals (titer > 50). This is not surprising since,

Fig. 4. Distal ilea from female New Zealand White rabbit. *a*: control animal (no surgery or immunization). *b* and *c*: rabbits with intestinal cannulas. Sections were taken several centimeters distal to the cannula tip immediately after the animal was killed (32 days post-operative). *d*, *e*, *f* and *g*: rabbits fitted with Thiry-Vella loops. Sections were taken transversely through the loop 32 days post-operative. Villi are broadened and blunted, and contain chronic lymphocytic inflammation of the lamina propria. Villous epithelium is cuboidal and degenerate. *f* and *g* show adhesions among damaged villi. HE  $\times$  150.

although mucosal lymphocytes traffic to a wide variety of mucosal tissues, the strongest response tends to be in the vicinity of the original antigen stimulation (Dunkley and Husband, 1990). In addition, a direct comparison of these titers is not particularly meaningful since the T-V loop secretions were used 'as-is', while feces were reconstituted in an arbitrary amount of buffer (10-fold volume/wet weight).

The most striking difference between the two methods was observed upon histologic examination of the tissues. The ileum distal to the insertion point of the cannula was indistinguishable from that of control tissue. In contrast, mucosa of the T-V loop was abnormal, with villus distortion and evidence of a chronic inflammatory process. Although the Peyer's patches themselves appeared normal, it is difficult to assess the influence of inflammatory mediators on either the induction of immune responses, or on the trafficking and terminal differentiation of lymphocytes within the lamina propria.

Anaerobic and aerobic bacteria were sampled from animals given either intestinal cannulas or T-V loops, and from untreated controls. Although there was substantial variability in the organisms cultured from each treatment group, it appeared that T-V loops were consistently colonized with large numbers of *Pseudomonas aeruginosa*. This bacterium was not recovered from any of the cannulated rabbits, but was present in lower numbers in two of four control animals. It seems reasonable that resident *Pseudomonas aeruginosa* may exploit the conditions of the T-V loops such that overgrowth occurs. The degree to which microorganisms are responsible for the degenerative changes observed in the T-V loops remains uncertain. Kern et al. (1987) treated T-V loops with antibiotics and observed significant villus atrophy in this 'quasi-sterile' environment, with no *Pseudomonas* present. This data supported earlier observations that duodenal trophic factors of pancreatic origin directly influence the size of villi (Altmann, 1971; Altmann and LeBlond, 1970). In either case, placement of an intestinal cannula did not appear to interfere with either the maintenance of 'normal' microflora, or exposure to chyme and trophic factors.

Taken together, our results indicate that a simple intestinal cannula is superior in many ways to the Thirty-Vella chronically isolated loops for delivery of

antigenic material to rabbit Peyer's patches. The single disadvantage to this approach is the inability to reliably remove intestinal secretions from the intestine for immunoassay. This problem was overcome by assaying feces for secretory antibodies. The simplicity of this approach may allow wider use of this model for studies of intestinal immunity.

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### References

- Altmann, G.G. (1971) Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. *Am. J. Anat.* 132, 167.
- Altmann, G.G. and LeBlond, C.P. (1970) Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. *Am. J. Anat.* 127, 15.
- Conner, M., Gilger, M., Estes, M. and Graham, D. (1991) Serologic and mucosal immune response to rotavirus infection in the rabbit model. *J. Virol.* 65, 2562.
- DeVos, T. and Dick, T.A. (1991) A rapid method to determine the isotype and specificity of coproantibodies in mice infected with *Trichinella* or fed cholera toxin. *J. Immunol. Methods* 141, 285.
- Dunkley, M.L. and Husband, A.J. (1990) Routes of priming and challenge for IgA antibody-containing cell responses in the intestine. *Immunol. Lett.* 26, 165.
- Fasano, A., Kay, B.A., Russel, B.G., Maneval, Jr., D.R. and Levine, M.M. (1991) Enterotoxin and cytotoxin production by enteroinvasive *Escherichia coli*. *Infect. Immun.* 58, 3717.
- Husband, A.J. and Lascelles, A.K. (1974) The origin of antibody in intestinal secretion of sheep. *Ajebak* 52, 791.
- Kabir, S. (1993) Simultaneous isolation of intestinal IgA and IgG from rabbits infected intraduodenally with vibrio cholerae 01 by combined lectin affinity chromatography involving jacalin and protein A. *Comp. Immunol. Microbiol. Infect. Dis.* 16, 153.
- Keren, D.F., Elliott, H.L., Brown, G.D. and Yardley, J.H. (1975) Atrophy of villi with hypertrophy and hyperplasia of Paneth cells in isolated (Thirty-Vella) ileal loops in rabbits. Light-microscopic studies. *Gastroenterology.* 68, 83.
- Keren, D.F., Holt, P.S., Collins, H.H., Gemski, P. and Formal,

- S.B. (1980a) Variables affecting local immune response in ileal loops: role of immunization schedule, bacterial flora and postsurgical inflammation. *Infect. Immun.* 28, 950.
- Keren, D.F., Scott, P.J. and Bauer, D. (1980b) Variables affecting the local immune response in Thiry-Vella loops II. Stability of antigen-specific IgG and secretory IgA in acute and chronic Thiry-Vella loops. *J. Immunol.* 124, 2620.
- Keren, D.F., Collins, H.H., Baron, L.S., Kopecko, D.J. and Formal, S.B. (1982) Intestinal immunoglobulin A responses in rabbits to a *Salmonella typhi* strain harboring a *Shigella sonnei* plasmid. *Infect. Immun.* 37, 387.
- Keren, D.F., Brown, J.E., McDonald, R.A. and Wassef, J.S. (1989a) Secretory immunoglobulin A response to Shiga toxin in rabbits: kinetics of the initial mucosal immune response and inhibition of toxicity in vitro and in vivo. *Infect. Immun.* 57, 1885.
- Keren, D.F., McDonald, R.A., Wassef, J.S., Armstrong, L.R. and Brown, J.E. (1989b) The enteric immune response to shigella antigens. *Curr. Top. Microbiol. Immunol.* 146, 213.
- Kern, S.E., Keren, D.F. and Pierson, C.L. (1987) Bacterial overgrowth and mucosal changes in isolated (Thirty-Vella) ileal loops in rabbits. Effects of intraluminal antibiotics. *Lab. Invest.* 57, 336.
- Kuhn, L.C. and Kraehenbuhl, J.P. (1979a) Role of rabbit secretory component, a secreted glycoprotein, in the specific uptake of IgA dimer by epithelial cells. *J. Biol. Chem.* 254, 11072.
- Kuhn, L.C. and Kraehenbuhl, J.P. (1979b) Interaction of rabbit secretory component with rabbit IgA dimer. *J. Biol. Chem.* 254, 11066.
- Kuhn, L.C. and Kraehenbuhl, J.P. (1981) The membrane receptor for polymeric immunoglobulin is structurally related to secretory component. Isolation and characterization of membrane secretory component from rabbit liver and mammary gland. *J. Biol. Chem.* 256, 12490.
- Majumdar, A.S. and Ghose, A.C. (1981) Evaluation of the biological properties of different classes of human antibodies in relation to cholera. *Infect. Immun.* 32, 9.
- Majumdar, A.S. and Ghose, A.C. (1982) Protective properties of anti-cholera antibodies in human colostrum. *Infect. Immun.* 36, 962.
- Mebus, C.A., Torres-Medina, A., Twiehaus, M.J. and Bass, E.P. (1976) Immune response to orally administered calf reovirus-like agent and coronavirus vaccine. *Dev. Biol. Standard* 33, 396.
- Meunier, L.D., Kissinger, J.T., Marcello, J., Nichols, A.J. and Smith, P.L. (1993) A chronic access port model for direct delivery of drugs into the intestine of conscious dogs. *Lab. Anim. Sci.* 43, 466.
- Porter, P., Kenworthy, R., Noakes, D.E. and Allen, W.D. (1974) Intestinal antibody secretion in the young pig in response to oral immunization with *Escherichia coli*. *Immunology* 27, 841.
- Rijpkema, S., Bik, E., Jansen, W., et al. (1992) Construction and analysis of a vibrio cholerae delta-aminolevulinic acid auxotroph which confers protective immunity in a rabbit model. *Infect. Immun.* 60, 2188.
- Sack, R.B., Johnson, J., Pierce, N.F., Keren, D.F. and Yardley, J.H. (1976) Challenge of dogs with live enterotoxigenic *Escherichia coli* and effects of repeated challenges on fluid secretion in jejunal Thiry-Vella loops. *J. Infect. Dis.* 134, 15.
- Silbart, L.K., Keren, D.F., McDonald, R.A., Lincoln, P.M., Goslinoski, L. and Smart, J.B. (1992) Characterization of the mucosal immune response to 2-acetylaminofluorene-protein conjugates. *Reg. Immunol.* 4, 245.
- Tadano, T., Kisara, K. and Stewart, J.J. (1992) A comparison of peripheral and central effects of clonidine on rat intestinal transit. *Res. Commun. Chem. Pathol. Pharmacol.* 78, 161.
- Wassef, J.S., Keren, D.F. and Mailloux, J.L. (1989) Role of M cells in initial antigen uptake and in ulcer formation in the rabbit intestinal loop model of shigellosis. *Infect. Immun.* 57, 858.