A Comparative Study of the Rabbit and Pig Gut Loop Systems for the Assay of Escherichia coli Enterotoxin

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ABSTRACT

A comparison was made between segments of pig and rabbit small intestine in their response to heat-labile (LT) and heat-stable (ST) preparations from porcine enteropathogenic Escherichia coli. Either whole cell lysates or dialysed broth culture supernatants were used as sources of LT and soft agar culture fluids as a source of ST. Whole cell lysates of all thirteen LT-producing E. coli strains tested regularly elicited fluid accumulation in rabbit gut loops. Whole cell lysates of certain E. coli strains considered to be nonenteropathogenic in pigs could also elicit a positive response in rabbit gut loops. When graded doses of LT were tested in pig and rabbit gut loops, the rabbit was more sensitive and is therefore considered preferable to the pig for quantitation of LT. In the rabbit, upper (jejunal) and lower (ileal) small intestine were compared for their response to LT and it was found that ileal loops were twice as sensitive but more prone to false positive reactions. When soft agar culture fluids of several enteropathogenic E. coli strains were tested in the rabbit, the response was inconsistent, and it was concluded that the rabbit is unsuitable for the assay of the heat-stable enterotoxin.

RÉSUMÉ

Cette étude visait à comparer la réaction de différents segments de l'intestin grêle de porcs et de lapins à l'injection de préparations thermolabiles (LT) et thermostables (ST) de cultures d'Escherichia coli entéropathogènes d'origine porcine. Les auteurs utilisèrent des lysats de colibacilles entiers ou le surnageant dialysé de cultures en bouillon, comme source de LT, et le liquide de cultures sur gélose semi-solide, comme source de ST. Les lysats cellulaires des 13 souches d'E. coli productrices de LT utilisées au cours de cette étude provoquèrent régulièrement une accumulation de liquide dans les anses intestinales des lapins. Les lysats cellulaires de certaines souches d'E. coli, considérées comme non-entéropathogènes, pouvaient aussi provoquer une réaction positive dans les anses intestinales des lapins. L'injection de doses graduées de LT dans des anses intestinales de porcs et de lapins révéla que ceux-ci étaient plus sensibles. En conséquence, ils semblent plus appropriés que les porcs pour la détermination quantitative de LT. En comparant le comportement de l'intestin grêle proximal (jéjunum) et distal (iléon) du lapin à l'endroit de LT, on réalisa que les anses iléales étaient deux fois plus sensibles, mais plus sujettes à donner de fausses réactions positives. L'injection aux lapins liquide des cultures sur gélose semi-solide de plusieurs souches entéropathogènes d'E. coli donna des résultats versatiles. Ceci amena les auteurs à conclure que le lapin ne constitue pas un animal satisfaisant pour l'étude de l'entérotoxine thermostable.

INTRODUCTION

In 1953, De and Chatterje (6) reported their use of the rabbit gut loop system for the investigation of *Vibrio cholerae*, the agent of cholera in man. Since then, the technique has become widely used for the study of bacteria which are associated with diarrhea in man and animals. In addition to *Vibrio cholerae* (6) and its enterotoxin (4,

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8), certain strains of *Shigella dysenteriae* (1, 14), *Clostridium perfringens* (9, 10), *Escherichia coli* (5, 12, 17, 18, 28), and *Pseudomonas aeruginosa* (15) have been shown to induce fluid accumulation in rabbit intestinal loops injected with the living organisms or their products.

The first reliable method of differentiating between enteropathogenic *E. coli* strains and nonenteropathogenic strains which are normally found in the digestive tract of pigs was provided by the gut loop technique performed in pigs (12, 19, 21). The use of ligated intestinal segments in pigs led to the demonstration of two forms of an enterotoxin which is elaborated by enteropathogenic *E. coli* (23). Although the ligated pig intestine is a reliable model for determining the enterotoxicity of living *E. coli* strains which cause diarrhea in swine (18, 21, 25), it is less suitable for extensive studies which require quantitation of the enterotoxin present in cell-free preparations. The marked variations in response from pig to pig along with the marked antero-posterior gradient in reactivity of the intestine are two of the main disadvantages (11).

Since there are several similarities between cholera in man and enteric colibacillosis in pig, gut loops in rabbits were investigated as a means of determining the enteropathogenicity of *E. coli* strains of porcine origin. However, rabbits were found to be unsatisfactory when living *E. coli* organisms were tested (26).

In this study, the rabbit gut loop model was investigated as a method for detection and quantitation of the cell-free enterotoxin produced by enteropathogenic *E. coli* strains isolated from pigs. Two areas of the rabbit intestine and both heat-labile and heat-stable forms of the *E. coli* enterotoxin will be tested.

MATERIALS AND METHODS

CULTURES

Twenty-one of the 22 *E. coli* strains which were used to produce cell-free preparations are listed in Tables I and II. The unlisted strain F11(P155) is an F11 strain which was conjugated with the porcine strain P155 from H. W. Smith (27) and now has the ability to produce both forms of the enterotoxin. Strains P307, P2050, P570, P568, P1108, P1253 and PAI had previously (11) been called G7, G205, E57, E68 type 1, G1108E, G1253 and Al respectively.

TABLE I. Selected Escherichia coli Strains Associated with Diarrhea of Pigs

Strain	Antigens		
	O	K	H
P307	8	87;88 a, b	19
638	8	87;88 a, c	?[c]
P2050	8	87;88 a, c	19
C662	138	81;88 a,c	19
P570[a]	138	81;	NM
P568	141	85;88 a, b	4
P1108	141	85;88 a, c	4
P1253	147	89;88 a, c	19
C105-62	147	?;88 a, c	19
C794-61	147	?;88 a, c	19
PAI	149	91;88 a, c	10
CS1522	149	91;88 a, c	10
V145	"116"[b]	"V17";88 a, c	43

[a]P570, associated with edema disease and post-weaning enteritis
[b]Related to antigens in quotation marks
[c]Antigen not known to author

Thirteen strains representing the *E. coli* serotypes most commonly associated with diarrhea in pigs, and previously shown to induce positive reactions when living cultures were injected into ligated segments of pig intestine (11), were used to produce enterotoxic preparations (Table I). All but strain P570 possess the K88 antigen, and except for strains 638, P1253 and CS1522, they all produced hemolysis on bovine blood agar.

Eight *E. coli* strains which are not associated with diarrhea of pigs were also selected for this study (Table II). Strains P400, P650, P1450, and CE38 had previously (11) been referred to as E4, E65, E145 and E38 respectively. All eight strains fail to produce the K88 antigen, and, except for strain F11, are hemolytic. Strain Cl produced a weak reaction when it is injected into pig gut loops (11); the other strains are negative.

CELL-FREE PREPARATIONS

Whole cell lysate (WCl) — The whole cell lysates were made from cells which were

TABLE II. Selected Escherichia coli Strains Not Associated with Diarrhea of Pigs

Strain	Animal Source	Antigen		
		O	K	H
M432	Healthy pig	139	82	1
P104	Pig with edema disease	139	82	?[a]
P400	Pig with edema disease	139	82	?
P650	Pig with edema disease	45	"E65"[b]	?
P1450	Pig with edema disease	141	85a, b,	4
C1	Calf with diarrhea	101	"RVC118"	?
CE38	Calf with septicemia	78	80	NM
F11	Fowl with bacteremia	18 ab	?	14

[a]Antigen not known to author
[b]Related to antigen in quotation marks

grown on trypticase soy agar (BBL) and disrupted by sonic oscillation. One litre Roux bottles, containing 150 ml of medium, were each inoculated with 5 ml of a 24 hour *E. coli* broth culture and incubated at 37°C for 24 hours. The growth was washed off with 5 ml of sterile distilled water and the cells in suspension were disrupted by treatment of 50 ml volumes for 30 minutes in an MSE 100 watt ultrasonic disintegrator[1]. The disrupted cells were separated from unbroken cells and large cellular debris by centrifugation at 30,000 x g for 20 minutes and filtration through 0.45μ cellulose acetate membrane filters. Dry weights were determined by incubation of 0.5 ml samples of clarified lysate preparations at 90°C for no less than 12 hours. Lysates obtained by this method of production contained approximately 33 mg of solids per ml. These preparations were either stored at –20°C or lyophilized and maintained at room temperature.

Broth Culture Supernatant (BS) — Broth culture supernatants were obtained by growing the *E. coli* organisms in a broth made of the PM-30[2] ultrafiltrate of a three per cent solution of proteose-peptone No. 3 (Difco), 0.5% sodium chloride, 1% phosphate buffer salt pH 7.4 (Harleco[3]), and 1% glucose. The broth was filtered through a sterile 0.45μ membrane filter and inoculated with 1/20th volume of an overnight broth culture. The inoculated culture was maintained under rigorous control of pH (7.4), temperature (37°C) and forced aeration, and growth was stopped by chilling when the culture had reached the end of the log phase. The cells were removed by centrifugation and filtration through a 0.45μ cellulose acetate membrane filter and the concentration of the culture medium constituents was considerably reduced by diafiltration of the broth culture supernatant on an XM-50 Diaflo membrane. After filtration of the dialysed XM-50 retentate through a 0.45μ cellulose acetate membrane filter, the final product which contained 0.7 to 0.8 mg of solids per ml was lyophilized and stored at room temperature.

Soft Agar Culture Fluid (SA) — Trypticase soy broth (BBL) was added to trypticase soy agar (BBL) to reduce the agar concentration to 0.36 per cent and glucose was added to a final concentration of 0.2 per cent. This semi-solid medium was dispensed in 150 ml volumes in 1 litre Roux bottles. Each bottle was then inoculated with 2 ml of a 24-hour broth culture, and incubated for 24 hours at 37°C. The bottles were cooled to 5°C and the fluid removed from the medium by gently squeezing through cheese cloth. The fluid was clarified by centrifugation at 20,000 x *g* for 20 minutes and filtration through 0.45μ cellulose acetate membrane filters. The clarified fluid was heated at 65°C for 15 minutes before the high molecular weight components were concentrated ten fold with PM-10 Diaflo membranes[4]. The preparations were stored at –20°C for no longer than three months prior to use.

[1]MSE 100 Watt ultrasonic disintegrator, Measuring Scientific Equipment Ltd., London, England.

[2]Diaflo membrane, Amicon Corporation, Lexington, Mass. U.S.A.

[3]Harleco, 60th Woodland Ave., Phila., Penn. 19143, U.S.A.

[4]The heat-stable enterotoxin is partially retained by these membranes.

Gut Loop Technique

Pig Gut Loop — Three to five week old pigs were tranquilized with acepromazine maleate (10 mg per animal) and general anesthesia was induced by the intravenous administration of a 2.5 per cent solution of sodium thiopental[5].

A laparatomy was performed to expose the anterior part of the small intestine and the first ligature was placed at a point on the intestine approximately 1 metre distal to the pyloric end of the stomach. Posterior to this ligature, twelve 12-15 cm loops separated by 4 cm interloops were prepared. Four ml samples were then injected with a 23 gauge needle and at least one negative and one positive control preparation were tested in each pig. The negative control was injected in the most anterior loop and the positive control in the most posterior loop since the reactivity of the small intestine was shown to decrease antero-posteriorly (11, 26).

The abdominal incision was closed, and 16-18 hr later the pigs were killed by electrocution and the small intestine removed. Fluid was collected by nicking the loops close to the ligature and the volume of fluid and the length of the segment were measured.

Rabbit Gut Loop — Each young adult rabbit (approx. 2 kg) was tranquilized by the intravenous injection of 2 mg of acepromazine maleate ten min prior to the intravenous administration of a 2.5 per cent solution of sodium thiopental. After the rabbit had been secured in dorsal recumbency, the small intestine was brought out of the peritoneal cavity through a midline incision and the intestinal lumen was flushed with warm saline.

Seven 10 cm loops separated by 2 cm interloops were prepared with ligatures. The first ligature was applied 55 cm from the pyloric end of the stomach wherever jejunal loops were used, whereas the ileal loops were located in the 85 cm portion of the small intestine just cranial to the common mesentery of ileum and appendix. In other respects, ileal and jejunal tests were similar. Two ml samples were injected using 25 gauge needles and at least one positive and one negative control preparation were included in each animal. After the intestine was brought back into the peritoneal cavity, the incision was closed with sutures.

Sixteen hours later, the rabbits were killed with pentobarbital and the test area immediately exposed. The volume of fluid was measured to the nearest 0.5 ml and the length of loops to the nearest 0.5 cm.

Interpretation

To minimize variations due to reactivity of different areas of the gut (20, 26) the positions of the loops receiving a given preparation were rotated in different animals. Two mg of neomycin sulfate were added to all sterile inocula to be injected in pigs to reduce interference by endogenous enteropathogenic *E. coli*. Animals were considered unreliable and the results discarded if the animals contained false positive loops or, in other words, if the negative control preparations produced more than 2 ml in rabbit gut loops and more than 8 ml in pig gut loops. On the other hand, animals were considered poorly reactive and the results discarded if the positive control preparations produced less than 5 ml in rabbit loops or less than 12 ml in pig loops. In order to minimize errors due to variation in loop length, reactions were recorded as the ratio of fluid in ml to length in cm, a procedure advocated by several workers using this technique (3, 11). Quantitation of *E. coli* enterotoxin was made according to the technique of Burrows and Musteikis (3). The toxin unit was estimated by determining the dose response curve to an enterotoxic preparation and interpolating the fifty per cent effective dose (ED_{50}). The dose response curve was obtained by plotting loop fluid volume to loop length ratios against the logarithm of the toxin concentration.

Comparison of the Pig and Rabbit Gut Loop Test Systems

Response to the labile toxin — Two fold serial dilutions of F11(P155) lysate from one batch were tested in jejunal loops of pigs and rabbits to determine the ED_{50} for both animal species. A total of ten pigs from two litters and 14 rabbits from one shipment were used for this purpose, and each dose was tested once in each animal.

The reliability of the rabbit intestinal loop model was assessed by testing lysates from enterotoxigenic and nonenterotoxigenic *E. coli* strains in jejunal loops of pigs and rabbits. The lysates in 50 mg doses

[5]Pentothal, Abbott Laboratories, Montréal, Qué.

were tested in 24 four to six week old pigs. This amount of toxin was used by Gyles (11) as a standard dose in pig jejunal loops. Jejunal tests in the rabbit were conducted by injecting enterotoxic and non-enterotoxic lysates in 20 and 60 mg doses, but only a limited number of the selected strains was tested at the higher dose.

Response to the stable toxin — The reactivity of pig and rabbit loops was investigated by comparing their response to soft agar culture preparations of several *E. coli* strains. A single dose containing the equivalent of 1.5 ml of ten times concentrated toxin was tested in 12 pigs and 20 rabbits.

Comparison of Jejunal and Ileal Test Areas in the Rabbit

Graded doses of a dialysed broth culture supernatant of *E. coli* strain F11(P155)

TABLE III. Enterotoxicity of Whole Cell Lysates[a] from Escherichia coli Strains Associated with Diarrhea of Pigs

	Jejunal Loop Response			
	Pig		Rabbit	
Strain	Pos.[b]/Total	Loop Fluid[c]	Pos./Total	Loop Fluid
P307	3/3	4.1 ± 1.7	22/23	1.6 ± 0.1
638	4/4	5.0 ± 1.1	5/5	2.0 ± 0.2
P2050	5/6	1.3 ± 0.3	4/4	1.7 ± 0.3
C662	6/6	3.6 ± 0.6	4/4	2.0 ± 0.3
P570	0/4	0.0 ± 0.0	3/6	0.3 ± 0.1
P568	4/4	3.4 ± 0.9	4/5	1.5 ± 0.3
P1108	8/8	4.1 ± 0.9	4/4	2.0 ± 0.4
P1253	4/4	4.0 ± 0.4	6/6	2.0 ± 0.2
C105	5/6	2.1 ± 0.6	7/7	1.4 ± 0.3
C794	4/4	2.9 ± 0.6	4/5	1.1 ± 0.3
PAI	6/6	4.1 ± 0.4	5/5	2.3 ± 0.2
CS1522	4/4	4.2 ± 1.0	4/4	2.1 ± 0.2
V145	4/8	1.8 ± 0.9	4/5	1.5 ± 0.4

[a]50mg/4ml/loop in pigs and 20 mg/2 ml/loop in rabbits
[b]Ratio of fluid to length greater than 0.2 in the rabbits or 0.5 in the pig
Mean ± standard error of the ratio of fluid in ml to length of loop in cm

TABLE IV. Enterotoxicity of Whole Cell Lysates[a] from Escherichia coli Strains Not Associated with Diarrhea in Pigs

	Intestinal Loop Resonpse			
	Pig		Rabbit	
Strain	Pos.[b]/Total	Loop Fluid[c]	Pos./Total	Loop Fluid
A. Strains of porcine origin				
M432	3/52	0.1	0/53	0.0
P104	1/5	0.2	11/36	0.3
P400	0/4	0.0	0/4	0.0
P650	0/6	0.0	0/19	0.0
P1450	1/4	0.3	3/7	0.6
B. Strains from sources other than pigs				
C1	0/6	0.0	0/13	0
CE38	2/5	0.3	3/8	0.4
F11	1/12	0.1	8/34	0.2

[a]50 mg/4 ml/loop in pigs and 20 mg/2 ml/loop in rabbits
[b]Ratio of fluid to length greater than 0.2 in the rabbit or 0.5 in the pig
[c]Mean of the ratio of fluid in ml to length of the loop in cm

were tested in ileal and jejunal segments of rabbit intestine. Two-fold serial dilutions of the LT preparation were tested in 24 rabbits from the same shipment; jejunal loop tests were conducted in 11 rabbits and ileal loop tests in 13 rabbits. The ED_{50} or toxin unit for each of the two areas was determined as described above.

Seven rabbits from another shipment were used to test increased challenge doses in the ileal portion of the small intestine. Finally, the specificity of both areas was also compared by testing cell-free preparations from two nonenterotoxigenic strains in 16 rabbits; jejunal tests were conducted in eight rabbits and ileal tests in the others.

TABLE V. Reactivity of Rabbit Jejunal Loops to 60 mg of Escherichia coli Lysate

Strain	Loop Response	
	Pos.[a]/Total	Loop Fluid[b]
M432	0/5	0.0 ± 0.0
P104	4/4	1.2 ± 0.2
P400	5/6	1.1 ± 0.2
P650	1/4	0.1 ± 0.1
P1450	4/6	0.7 ± 0.2
C1	0/6	0.0 ± 0.0
CE38	5/5	1.1 ± 0.1
P570	0/8	0.0 ± 0.0

[a]Loops containing more than 0.2 ml of fluid per cm of loop
[b]Mean ± SE of the ratio of fluid in ml to length of the loop in cm

RESULTS

Comparison of the Pig and Rabbit Gut Loop Test Systems

Heat-labile enterotoxin — Lysates from all the strains associated with diarrhea in pigs, except strain P570, regularly elicited a positive response in both pigs and rabbits (Table III). Lysates from strains PA1, CS1522, P1253, P1108 and 638 produced strong reactions in both animal species, whereas lysates from strains V145, C105 and C794 were less active in both rabbits and pigs. The lysate from strain P570 did not elicit a response in the pigs, but a weak response was obtained in the rabbits (three of the six test loops giving responses of 0.3, 0.8 and 0.9 ml of fluid per cm). Lysate from strain P2050 gave the lowest mean response in the pig, but a good response was obtained in the rabbit loops. When similar preparations from strains not associated with diarrhea in pigs were tested, they all failed to elicit any response in the pigs, but preparations of strains P104, P1450, CE38 and F11 induced positive loops in the rabbits (Table IV). When the lysates which were nonenterotoxic in pigs were tested in rabbits at a dose of 60 mg, strains P104, P400, P450 and CE38 regularly elicited a positive response, whereas strains M432, C1 and P570 never produced a positive reaction (Table V).

The sensitivity of rabbit jejunal loops to the heat-labile enterotoxin was compared to that of pig jejunal loops by testing graded doses of *E. coli* F11(P155) lysate in both species. In pigs, doses ranging from 1.25 mg to 80 mg were tested, and a maximum response was obtained with 40 and 80 mg. A dose response curve was drawn and the ED_{50} estimated (Fig. 1). In rabbits, doses ranging from 0.125 mg to 8.0 mg were tested, and a maximum response was obtained with 2.0, 4.0 and 8.0 mg of lysate. The ED_{50} which was estimated from the dose response curve was 0.40 mg (Fig. 2).

Heat-stable enterotoxin — The results of soft agar culture fluids are shown in Table VI. Four of the 20 rabbits were excluded because of false positive responses. All the preparations from the nine strains associated with diarrhea in pigs consistently elicited a positive response in pigs, with those strains P570 and P1108 being the most active. The same preparations produced inconsistent reactions in rabbits, with those strains P2050, C662, P1253 and C105 failing to elicit a response and strain P570 producing maximum response but irregularly. The mean fluid response of all the positive loops injected with soft agar preparations was 2.0 ml per cm. The toxins of non-pathogenic strains C1 and P650 were negative except for one weak reaction in a pig.

Comparison of Jejunal and Ileal Test Areas in the Rabbit

One rabbit in which jejunal tests were conducted was discarded because of erratic results and two of the rabbits used for ileal tests were rejected because they gave positive responses to 2.0 mg of the F11 broth culture supernatant. More fluid per unit length of intestine accumulated in ileal loops compared with jejunal loops and the maximum mean response was 2.2 ml per cm

in the ileal loops and 1.4 ml per cm in the jejunal tests. Figure 3 shows the dose response curves which were used to calculate the ED_{50}. The toxin unit for the ileum was 0.30 mg compared to 0.35 mg for the jejunum.

The specificity of both areas was studied by injecting cell-free preparations from nonenterotoxigenic strains of *E. coli* (Table VII). False positive reactions were observed in both areas, but were more frequent in the ileal loops, particularly when a broth culture supernatant of strain F11 was injected. Forty ileal loops were injected with negative preparations and 15 of these responded with over 0.2 ml of fluid per cm, having an average of 1.3 ml of fluid per cm. On the other hand, only seven of the 40 jejunal tests contained more than 0.2 ml of fluid per cm, and a mean response

TABLE VI. Enterotoxicity of Soft Agar Culture Fluids[a] from Escherichia coli Strains

	Intestinal Loop Response			
	Pig		Rabbit	
Strain	Pos.[b]/Total	Loop Fluid[c]	Pos.[b]/Total	Loop Fluid
A. Strains associated with diarrhea in pigs				
P2050	4/4	2.0 ± 0.8	0/2	0.0 ± 0.0
C662	5/6	1.4 ± 0.5	0/3	0.0 ± 0.0
P570	4/4	5.1 ± 0.3	4/5	1.6 ± 0.6
P1108	5/5	4.6 ± 0.9	2/4	1.0 ± 0.6
P1253	4/4	1.5 ± 0.2	0/3	0.0 ± 0.0
C105	4/4	1.7 ± 0.3	0/3	0.0 ± 0.0
PAI	9/10	3.3 ± 0.6	4/7	1.1 ± 0.4
CSI522	10/10	3.5 ± 0.4	1/4	0.7 ± 0.6
V145	3/4	3.1 ± 1.0	1/3	0.5 ± 0.5
B. Strains not associated with diarrhea in pig				
C1	1/4	0.2 ± 0.2	0/5	0.0 ± 0.0
P650	0/4	0.0 ± 0.0	0/4	0.0 ± 0.0

[a]1.5 ml of SA fluid concentrated ten times on a PM-10 Diaflo membranes (Amicon Corp., Lexington, Mass.) were injected in pig and rabbit loops
[b]Ratio of fluid to length greater than 0.2 in the rabbit or 0.5 in the pig
[c]Mean ± SE of the ratio of volume in ml to length in cm

TABLE VII. Reactivity of Different Areas of the Rabbit Small Intestine to Escherichia coli Cell-free Preparations

		Loop Response			
		Jejunum		Ileum	
Preparation	Dose mg	Pos.[a]/Total	Loop Fluid[b]	Pos.[a]/Total	Loop Fluid
M432 WCL[c]	20	0/8	0.0 ± 0.0	2/8	0.2 ± 0.2
F11 WCL	20	4/8	0.4 ± 0.2	3/8	0.3 ± 0.2
F11 BS[d]	2	2/8	0.2 ± 0.2	3/8	0.4 ± 0.2
	4	0/8	0.0 ± 0.0	3/8	0.6 ± 0.3
	8	1/8	0.1 ± 0.1	4/8	1.0 ± 0.4
F11 (P155) BS	2	8/8	1.6 ± 0.1	8/8	2.2 ± 0.1

[a]Ratio of fluid to length greater then 0.2 in the rabbit and 0.5 in the pig
[b]Mean ± SE of the ratio of fluid in ml to length of loop in cm
[c]Whole cell lysate
[d]Broth culture supernatant

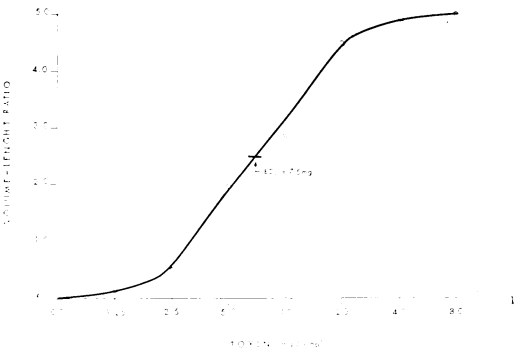

Fig. 1. Dose response curve for Escherichia coli F11 (P155) lysate tested in pigs. The curve is given by plotting the loop response (ml of fluid per cm of intestine length) against the logarithm of the toxin concentration.

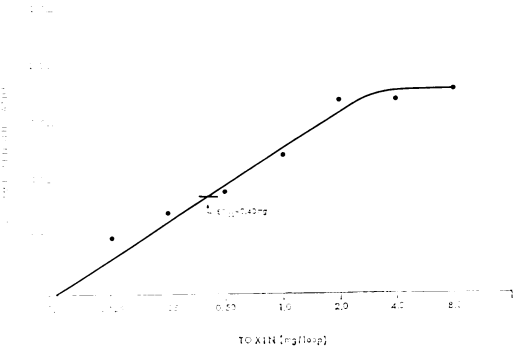

Fig. 2. Dose response curve for Escherichia coli F11 (P155) lysate tested in rabbits. The curve is given by plotting the loop response (ml of fluid per cm of intestine length) against the logarithm of the toxin concentration.

of 0.8 ml of fluid per cm was produced. When the numbers of false reactions occurring in each rabbit were considered, only one of the eight rabbits had more than one false reaction in the jejunal tests, whereas four of the eight used for ileal tests had more than one false reaction. The positive toxin control preparation consistently elicited a positive response in all 16 rabbits with mean responses of 2.2 ml of fluid per cm in the ileal loops and 1.6 per cm in the jejunal loops.

Throughout this study, approximately 30 per cent of the rabbits used in jejunal tests did not survived 16 hours following surgery, whereas most of the rabbits used in ileal tests did. However, the results in the animals which died earlier than 16 hours were considered if the controls were satisfactory.

DISCUSSION

By testing living organisms in ligated intestinal segments, Smith and Halls (25) showed that rabbits were less reliable than pigs for demonstration of enteropathogenicity of *E. coli* strains of porcine origin. In this study, ligated intestinal segments of rabbits were used to test cell-free preparations of similar strains as the failure of the rabbit system to demonstrate enteropathogenicity could have been related to the inability of the microorganisms to produce the toxin *in situ*. The rabbit system was found to be more sensitive to the heat-labile enterotoxin than the pig intestinal loop system (Figs. 1 and 2). Similar results have recently been obtained by other workers (20, 24). One pig gut loop unit was equivalent to 18.7 rabbit jejunal units. All the lysates which elicited a positive response in pig gut loops produced fluid in rabbit jejunal loops (Table III).

The rabbit test system is less specific than that of the pig since preparations of certain strains not associated with diarrhea in pigs elicited a positive rabbit jejunal loop response similar to that of enteropathogenic strains. The occurrence of the positive reactions was limited to given preparations (Table IV), and their incidence could be increased with a higher dose (Table V). These "false" positive reactions appear to

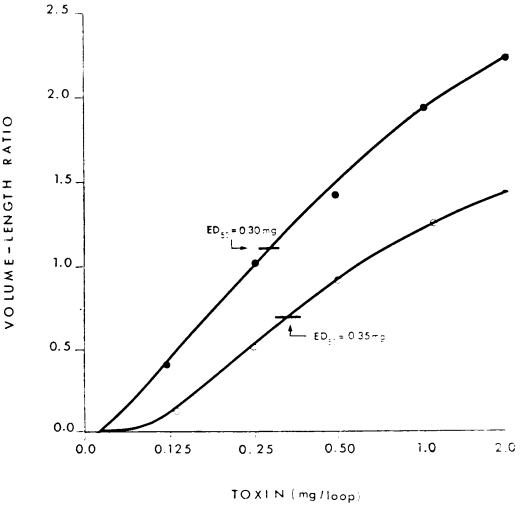

Fig. 3. Dose response curves for Escherichia coli F11 (P155) dialysed broth culture supernatant injected into jejunal loops (0) and ileal loops (•) of the rabbit. The curves are given by plotting the loop response (ml of fluid per cm of intestine length) against the logarithm of the toxin concentration.

differ from those observed in the rabbit ileal loops by other workers (1, 3, 7, 20) as the latter reactions were independent of the injected preparation (3) and could be eliminated by avoiding the lower ileum (20). A possible explanation for these "false" positive responses would be that these strains produce minimal amounts of enterotoxin which were not detected in the less sensitive pig gut loop system. This hypothesis suggests that enterotoxin production in *E. coli* strains may be similar to that in *V. cholerae* type E1 tor in which certain strains can produce minimal amounts of enterotoxin although they are not considered as pathogens (2, 24). Another explanation would be that these strains produce a different enterotoxin which, as the enterotoxin from the calf strains (26), does not elicit a response in the pig intestine.

The limited studies with the heat-stable enterotoxin confirm the reports that the rabbit gut is less sensitive to ST than the pig gut (20, 24). This was supported by the fact that only the most active preparations, as determined in pigs, elicited positive loops in rabbits (Table VI). Furthermore, it is suggested that there is a threshold in the potency below which no response to ST is observed since the positive rabbit jejunal loops injected with ST were distended maximally.

Gyles (14) found that although strain P570 gave strong reactions in tests of the living culture, lysate of that strain failed to produce positive loops. The present study has confirmed that P570 lysate does not contain any detectable enterotoxic activity (Table V). However, the soft agar preparation of P570 was very active in both pig and rabbit gut loops (Table VI). The fact that *E. coli* strain P570 produces ST only is in agreement with the observation of Smith and Gyles who state that the LT component was produced by only those wild strains which possessed the K88 antigen (23).

Because the upper part of the pig small intestine is more reactive to the *E. coli* enterotoxin than its lower part (11, 26) and because the lower ileum of the rabbit is more reactive to the vibrio enterotoxin than the upper ileum (16), gut loop tests to *E. coli* enterotoxin were conducted in the upper ileum and the lower ileum of rabbits. The present study indicates that, as with the vibrio enterotoxin, the lower ileum (ileal tests)of the rabbit is more reactive to the heat-labile enterotoxin of *E. coli* than the so-called upper ileum (jejunal tests). In this study, although only 1.2 ileal toxin unit was necessary to produce half maximum reaction in jejunal tests, 250 μg of LT elicited twice as much fluid in the ileal tests as in the jejunal tests (Fig. 3). This apparent discrepancy is due to the fact that the maximum reaction observed in jejunal tests is about 70 per cent of that observed in ileal tests. On the other hand, by defining the toxin unit as the amount of toxin necessary to produce 1.0 ml of fluid per cm in rabbits, the toxin unit in jejunal tests would become the equivalent of 1.8 ileal toxin units, and the difference in sensitivity would become more obvious. The increase in sensitivity of the ileal tests was shown to be associated with a decrease in specificity since the occurrence of false positive reactions was more common in the ileal than in the jejunal tests (Table VII).

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Book Review

MEDICAL AND VETERINARY PROTOZOOLOGY. AN ILLUSTRATED GUIDE. *K. M. G. Adam, J. Paul and V. Zaman. Published by Longman Canada Limited, Don Mills, Ontario. 1971. 200 pages. Price $21.50.*

As the authors state in their preface, this book is primarily a guide to the identification of protozoa which cause disease in man and domestic animals. In keeping with this objective it contains many more pages of excellent illustrations than written text.

The book consists of 200 pages with chapters on lumen dwelling and intracellular parasites of the alimentary tract, intracellular tissue parasites, Trypanosomes, malaria parasites and intracellular blood and tissue parasites. Special chapters are devoted to pathogenic free living amoeba, arthropod vectors and laboratory aids in the diagnosis of protozoan infections. A useful series of appendices give a skeleton classification of the protozoa and explain various technical manipulations such as phase contrast microscopy, sterilization and disinfection.

There are 186 figures, most of them original photographs and 126 of them are in color. These color photographs, especially those of blood protozoa and histological sections add to the excellent quality of the illustrations and enhance the value of the book in the field of diagnostic protozoology.

For the identification of the protozoan parasites of man and most of those occurring in domestic animals this text and its illustrations form an invaluable guide. However, in a few areas related specifically to veterinary protozoology, such as the coccidia, it is only of limited value. — *M. A. Fernando.*