





**Intestinal “Flora”**

# Introduction to the Enterobacteriaceae

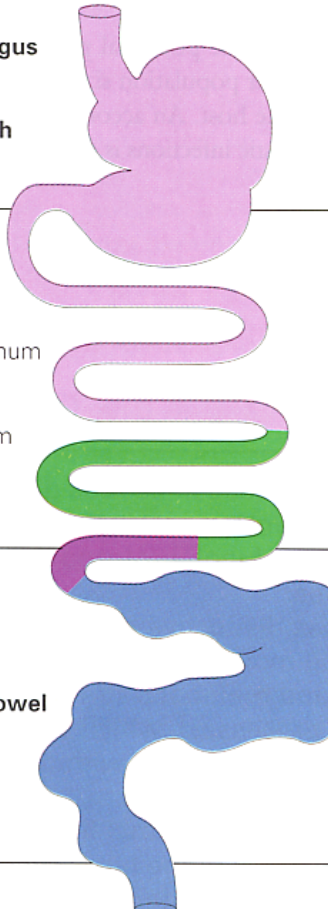
The concentration and population of microorganisms that constitute the normal intestinal flora vary with the location along the intestine

•predominant species are anaerobes





•most common aerobe is *E. coli* (conc. only 1/1000 of anaerobes in colon)

•the normal flora exert a profound influence on intraluminal constituents: food, urea, bilirubin, bile salts, drugs and potential toxins.





We get 10% of our total energy from colonic fermentation of our food

density	frequency of occurrence in population				
esophagus stomach 	lactobacilli <i>Helicobacter pylori</i>				
small bowel duodenum jejunum ileum	lactobacilli streptococci Enterobacteria <i>Bacteroides</i> spp.				
large bowel	<table border="0"> <tr> <td><i>Bacteroides</i> spp. <i>Fusobacterium</i> spp. <i>Strep. faecalis</i> <i>Escherichia coli</i></td> <td>enterobacteria <i>Klebsiella</i> spp. eubacteria bifidobacteria</td> </tr> <tr> <td>lactobacillus <i>Staph. aureus</i> <i>Clostridium</i> spp.</td> <td>streptococci <i>Pseudomonas</i> <i>Salmonella</i></td> </tr> </table>	<i>Bacteroides</i> spp. <i>Fusobacterium</i> spp. <i>Strep. faecalis</i> <i>Escherichia coli</i>	enterobacteria <i>Klebsiella</i> spp. eubacteria bifidobacteria	lactobacillus <i>Staph. aureus</i> <i>Clostridium</i> spp.	streptococci <i>Pseudomonas</i> <i>Salmonella</i>
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fecal material	<table border="0"> <tr> <td><i>Bacteroides</i> spp. bifidobacteria eubacteria</td> <td>coliforms <i>Strep. faecalis</i></td> </tr> </table>	<i>Bacteroides</i> spp. bifidobacteria eubacteria	coliforms <i>Strep. faecalis</i>		
<i>Bacteroides</i> spp. bifidobacteria eubacteria	coliforms <i>Strep. faecalis</i>				

density

very low ( $10^3$ - $10^5$ /g)	
low ( $10^5$ - $10^8$ /g)	
medium ( $10^8$ - $10^{10}$ /g)	
high ( $>10^{10}$ /g)	

frequency

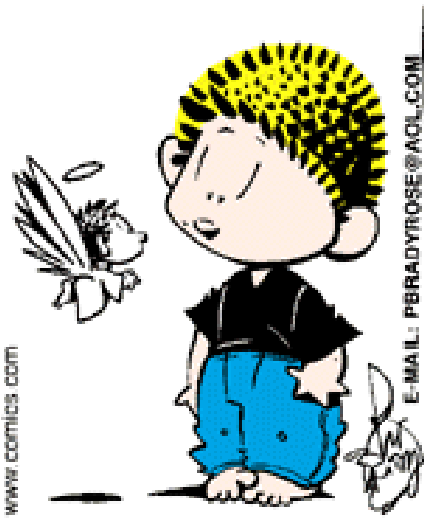
<10%	
10-25%	
25-75%	
100%	



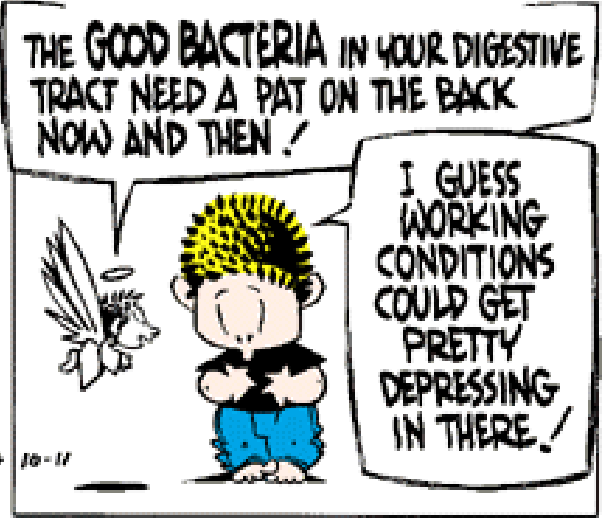
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# The Family Enterobacteriaceae

- A large number of Genera have now been established by DNA studies.
- In the main, the common human pathogens are restricted to the
  - *Escherichia*
  - *Shigella*
  - *Salmonella*
- The Many Other Members of this Family are Found in Human Infections but Usually as Opportunists in the Setting of an Underlying Illness.

**APPENDIX 20-1. GENERAL CHARACTERISTICS OF SOME ENTEROBACTERIACEAE**

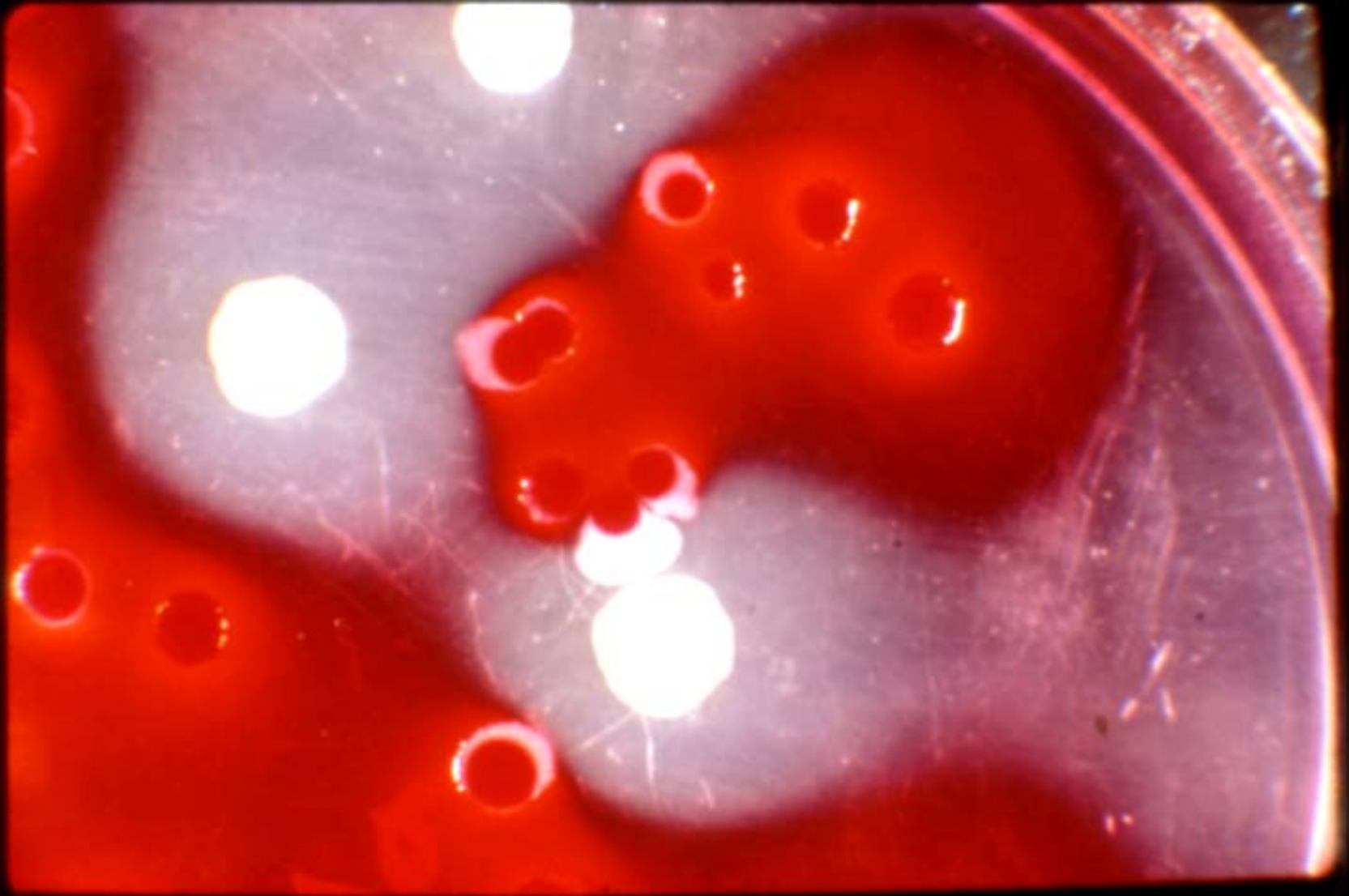
Organism	Serologic Type(s) (Antigens)	Bacteriologic Features						Major Disease(s)	Found in Normal Flora
		Lactose	Indole	Urease	Hydrogen Sulfide	Motility	Other		
<i>Escherichia coli</i>	150+ (O, K, H)	+	+	-	-	+		Urinary tract infections; diarrhea; opportunistic	Yes
<i>Shigella dysenteriae</i>	10 (O)	-	v	-	-	-		Dysentery (type 1, severe)	No
<i>Shigella flexneri</i>	6 (O)	-	-	-	-	-		Dysentery	No
<i>Shigella boydii</i>	15 (O)	-	v	-	-	-		Dysentery	No
<i>Shigella sonnei</i>	1 (O)	-	-	-	-	-		Dysentery	No
<i>Klebsiella pneumoniae</i>	72 (K)	+	-	+ <sup>a</sup>	-	-	Encapsulated	Pneumonia; opportunistic	Yes
<i>Enterobacter</i> sp.		+	-	-	-	+		Opportunistic	Yes
<i>Serratia marcescens</i>		-	-	v <sup>a</sup>	-	+	Red pigment	Opportunistic	Yes
<i>Salmonella</i> serotypes	1500+ (O, H)	-	-	-	+	+		Diarrhea	No
<i>Salmonella choleraesuis</i>	1 (O, H)	-	-	-	v	+		Bacteremia	No
<i>Salmonella typhi</i>	1 (O, H, K)	-	-	-	+	+		Enteric (typhoid) fever	No
<i>Salmonella paratyphi A.</i>	(O, H)	-	-	-	+	+		Diarrhea; enteric fever	No
<i>Citrobacter</i> sp.		v	-	v <sup>a</sup>	+	+		Opportunistic	Yes
<i>Proteus mirabilis</i>		-	-	+	+	+	Swarming <sup>b</sup>	Opportunistic	Yes
<i>Proteus vulgaris</i>		-	+	+	+	+	Swarming <sup>b</sup>	Opportunistic	Yes
<i>Morganella morganii</i>		-	+	+	-	+		Opportunistic	Yes
<i>Providencia</i>		-	+	v	-	+		Opportunistic	Yes
<i>Yersinia pestis</i>		-	-	-	-	-		Plague	No
<i>Yersinia pseudotuberculosis</i>	10 (O, H)	-	v	+ <sup>c</sup>	-	+ <sup>c</sup>		Mesenteric lymphadenitis	No
<i>Yersinia enterocolitica</i>	50+ (O, H)	-	-	+ <sup>c</sup>	-	+ <sup>c</sup>		Mesenteric lymphadenitis; enteric fever; diarrhea	No

Abbreviations: + = more than 90% of strains positive; - = less than 10% of strains positive; v = variable (some strains positive, others negative).

<sup>a</sup> Positive reactions weak or delayed compared to those of Proteeae.

<sup>b</sup> Growth swarms over surface of agar plates.

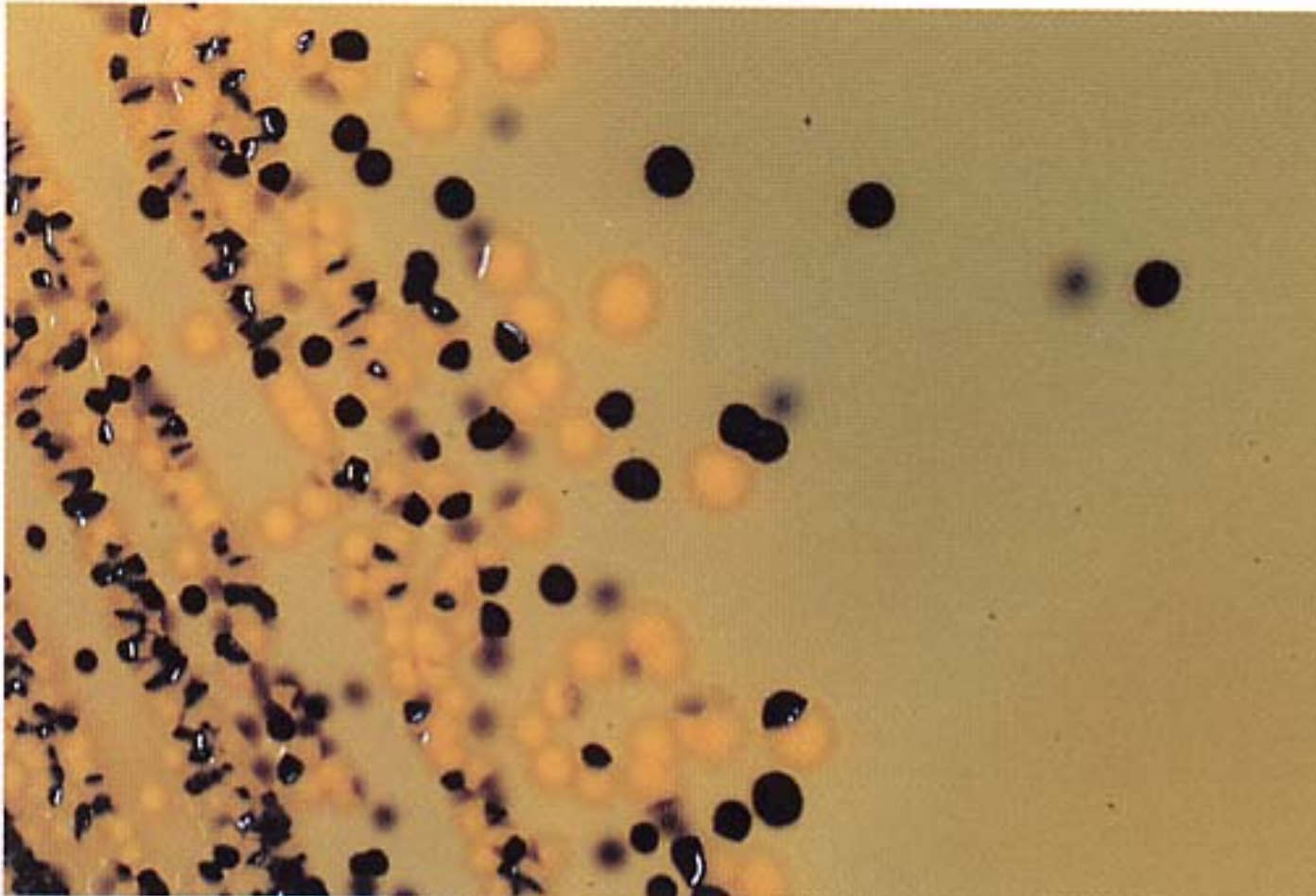
<sup>c</sup> Positive reactions seen at 25°C but not at 37°C.



EMB agar - *E. coli* w/ metallic sheen  
Disadvantage is that *Proteus* swarms



MacConkey agar is used in many laboratories to isolate enteric bacilli. The agar medium contains an inhibitor of gram positive bacteria, bile salts, lactose and a pH indicator. The fermentation of lactose by *E. coli* (left panel) is seen by dark pink colonies while non-lactose fermenting bacteria (right-hand panel) appear as colorless colonies. The distinction between lactose and non-lactose fermentation is based on an old concept in medical bacteriology that lactose fermenting species like *E. coli* did not cause enteric disease while non-fermenting species like *Salmonella* and *Shigella* caused most diarrheal disease. Nevertheless it is still a useful tool in the diagnostic laboratory



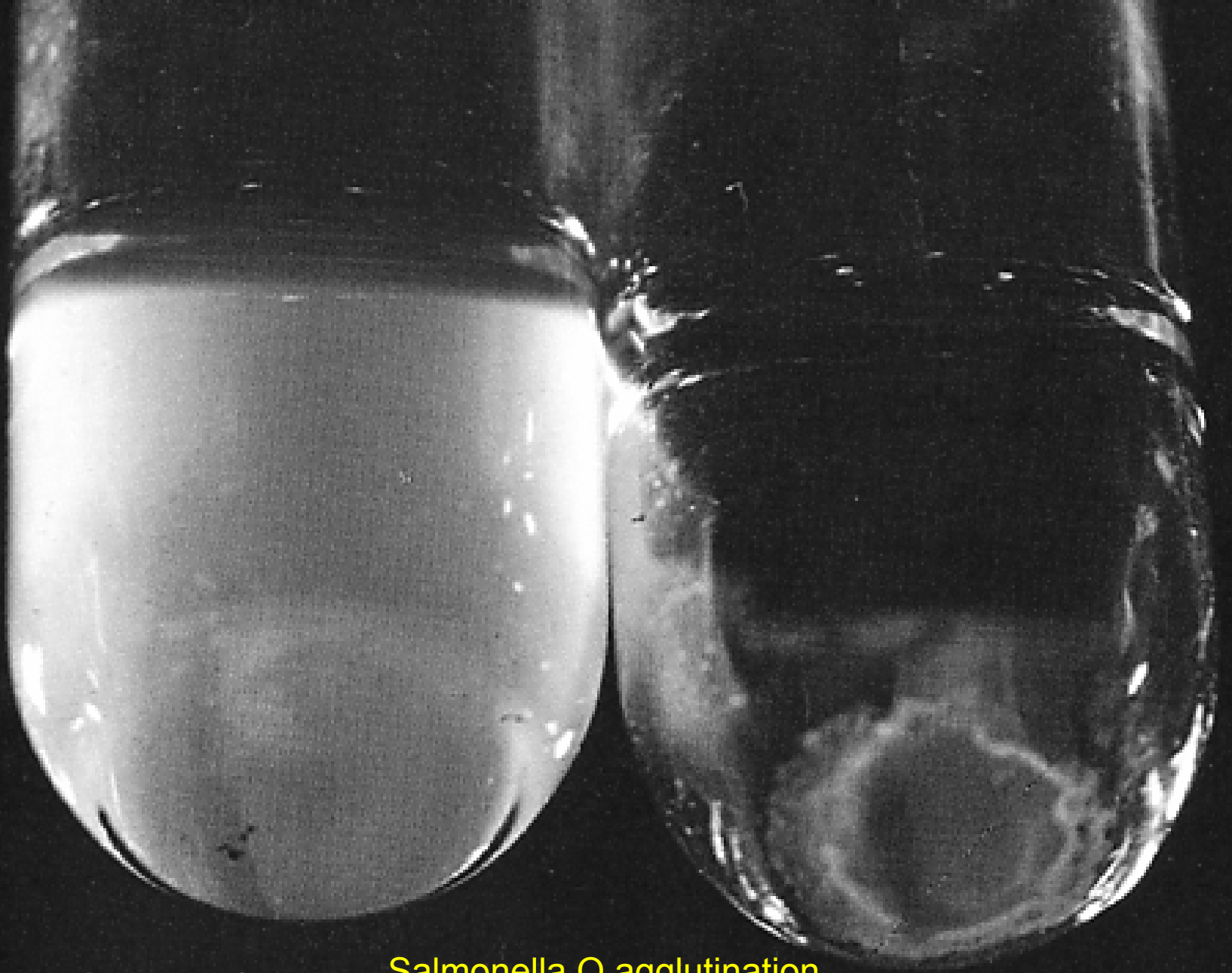
A large number of differential media have been developed for use in the clinical laboratory. One of these, Hektoen agar is illustrated here. Lactose, sucrose and salicin-fermenting organisms appear yellow, non-fermenters appear colorless and  $H_2S$  production turns the colonies black.



Some bacteria give a distinctive appearance on agar medium. For examples, some species of *Proteus* spread as a film often extending from the site of inoculation as waves of growth.



The biochemical identification of Bacteria has been automated and miniaturized. Here is the API 20 strip is shown. It is a self-contained system of 20 microtubes which is used as a miniaturized version of conventional procedures. Numerical coding of results allows computerized interpretations for identification of groups and species of organisms.



Salmonella O agglutination

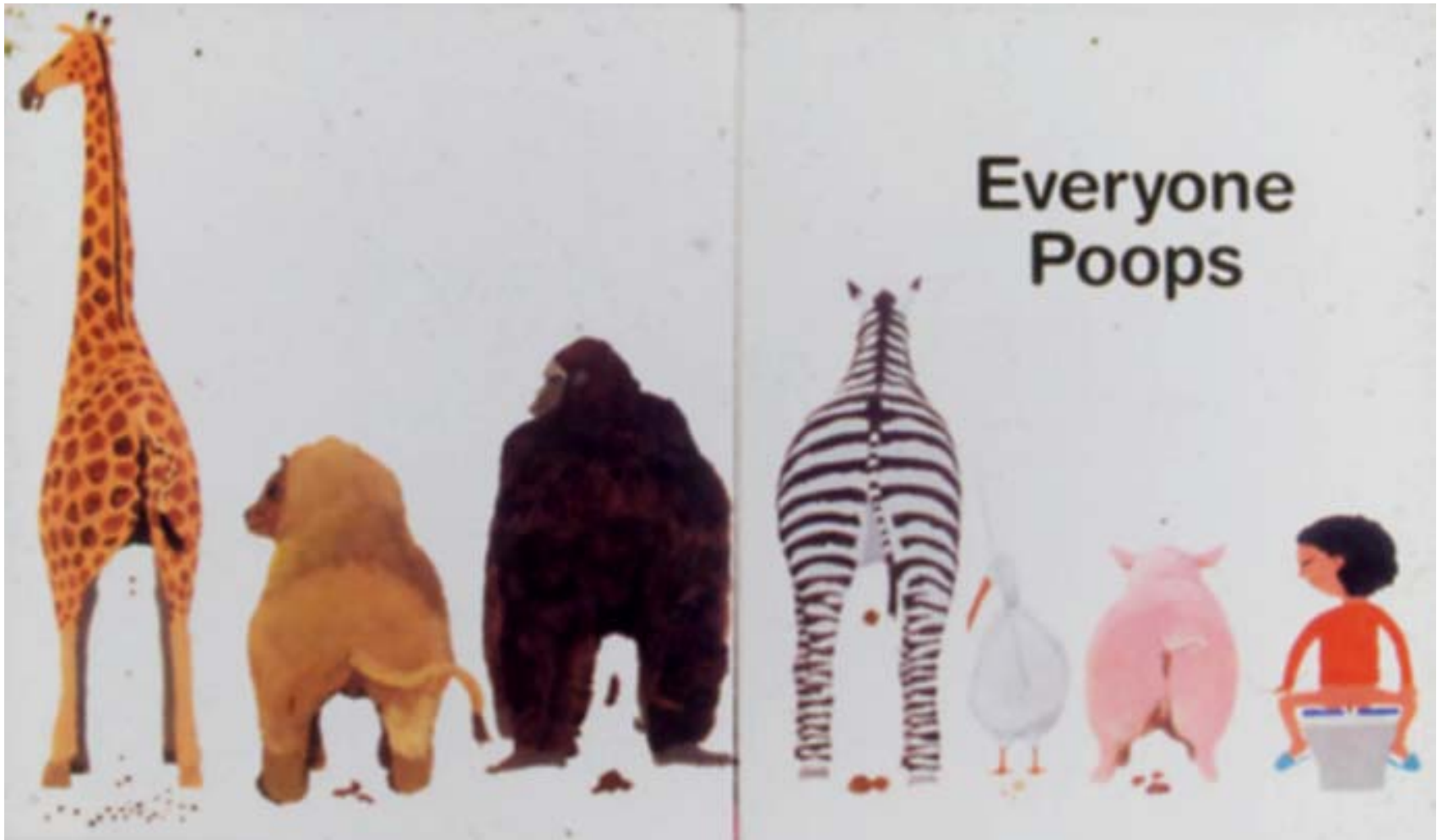
E. coli API strip



Salmonella API strip



# What is Diarrhea and What Causes It?



With a complaint of "diarrhea," the physician must establish if this represents a change in the patient's bowel habit and if the complaint arises from a perception of increased frequency of stool, increased volume or both.

**Value and Mean Composition of Fecal Losses of Water and Electrolytes in Normal or Diarrheal Stools of an Infant**

	Normal stools	Acute diarrhea
Water (ml/day)	20-30: 3 ml/kg	200-400: 5 to 20 ml/kg
Na (mmol/L)	1.3	50 to 100
K (mmol/L)	4	20 to 50
CL (mmol/L)	0.6	20 to 50
HCO <sub>3</sub> (mmol/L)	—	15 to 25

**If the diarrhea is acute (i.e., lasting less than two weeks), the malabsorption of fluid and electrolytes probably has an infectious or toxic cause.**

When diarrhea lasts for a longer period of time, other explanations need to be considered.

The four most common causes of chronic diarrhea are

- (1) the irritable bowel syndrome
- (2) inflammatory bowel disease
- (3) malabsorption
- (4) carcinoma of the colon.

# Acute Bacterial Diarrheas can be Classified into 2 Types

**Toxigenic-** enterotoxin is the major pathogenic mechanism  
-cytotoxic (producing intestinal fluid secretion by activation of intracellular enzymes w/o damage to epithelial surface)  
-cytotoxic (causing injury to the enterocyte as well as inducing fluid secretion)

**Invasive-** organism penetrates the enterocyte as primary event  
although an enterotoxin may be produced as well

**TABLE 74–1 ■ Clinical Features of Acute Diarrhea**

CLINICAL OBSERVATION	ANATOMIC CONSIDERATION	PATHOGENS TO CONSIDER
Passage of few, voluminous stools	Diarrhea of small bowel origin	<i>Vibrio cholerae</i> , enterotoxigenic <i>Escherichia coli</i> , <i>Shigella</i> strains early in the infection, <i>Giardia</i>
Passage of many small-volume stools	Diarrhea of large bowel origin	<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , <i>Entamoeba histolytica</i>
Tenesmus, fecal urgency, dysentery	Colitis	<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , <i>E. histolytica</i>
Vomiting as the predominant symptom	Gastroenteritis	Viral agents ( <i>Rotavirus</i> , Norwalk virus) or intoxication ( <i>Staphylococcus aureus</i> , <i>Bacillus cereus</i> )
Fever as a predominant finding	Mucosal invasion	<i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> , viral agents (rotavirus, Norwalk virus)

# Three Major Clinical Syndromes caused by Bacterial Infections

## 1) Food Poisoning

*Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens* and *Bacillus cereus* are responsible for 90% of these outbreaks.

## 2) Infectious Gastroenteritis

We will learn about:

*E. coli*, *Shigella*, *Listeria*, *Salmonella*, *Vibrio cholera*, *Campylobacter*,  
*Helicobacter pylori*

## 3) Traveler's Diarrhea



**Epidemics**

500 cases EHEC  
370,000 cryptosporidiosis  
500,000 cholera

**Traveler's diarrhea**

> 5 million cases worldwide, rarely fatal

**Food poisoning**

> 12,000 cases/year reported in the United States, ~6.5 million unreported

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**Impaired hosts**

50%–90% of 3 million AIDS patients worldwide annually

**Institutions**

> 560,000 cases in the United States

**Endemic diarrhea**

2–12 illnesses/child/yr  
9000–12,000 deaths/day worldwide

Mild Dehydration  
5% or < body  
Weight lost




Irritability  
Skin pale but  
lips may be vivid  
pink from  
Hemoconcentration.

Moderate Dehydration  
Up to 10% body weight  
lost  
Pallor is striking  
Initial Irritability gives way  
to listlessness  
Sunken eyes from loss of  
water from retro-orbital  
fat pad

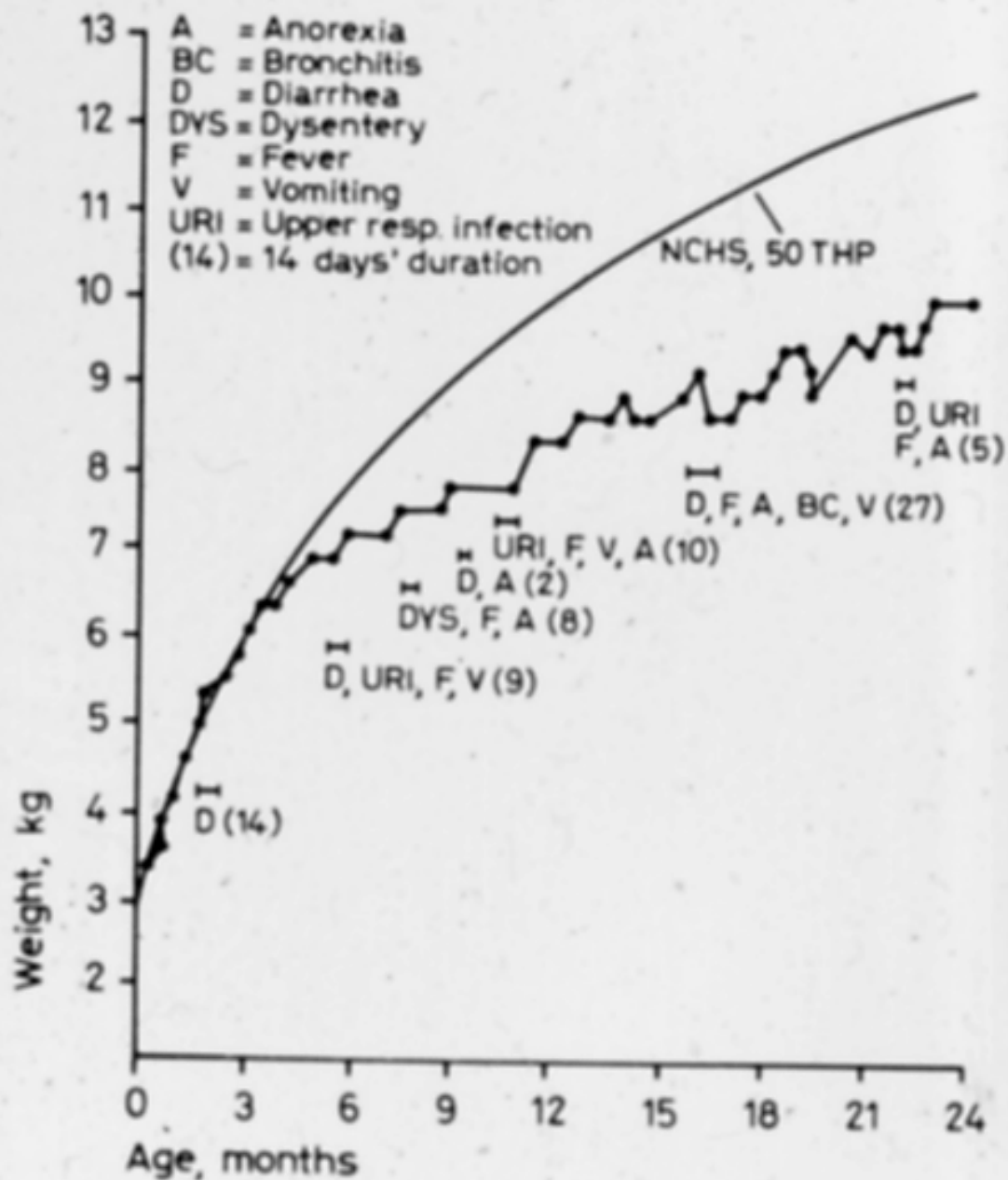


Loss of skin turgor and  
Elasticity





Severe dehydration  
10-15% of body weight loss  
Oliguria is invariably present  
BUN rises; acidosis usually present  
Mortality approaches 50%.



# Stop Trot

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Oral rehydration salt solutions

## **Bicarbonate solution**

Dissolve in 1 liter of potable water:

	<b>grams</b>
Sodium chloride	3.5
Sodium bicarbonate (Sodium hydrogen carbonate)	2.5
Potassium chloride	1.5
Glucose, anhydrous	20.0

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## **Citrate solution**

Dissolve in 1 liter of potable water:

	<b>grams</b>
Sodium chloride	3.5
Trisodium citrate, dihydrate	2.9
Potassium chloride	1.5
Glucose, anhydrous	20.0

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**Reduced mortality 50% →→ <10%**

# *ESCHERICHIA COLI*

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- ◆ **Gram negative Enterobacteriaceae**
- ◆ **Commensal of intestinal tract of man and animals**
- ◆ **Some *E.coli* produce severe disease in man and animals**
  - Pathogenic *E. coli* contain Pathogenicity Islands
- ◆ **Disease in man**
  - **Extraintestinal infections**
    - » UTI, Septicaemia & meningitis (in newborns)
  - **Intraintestinal infections**
    - » Infantile diarrhoea, dysentery, haemorrhagic colitis, traveller's diarrhoea & protracted diarrhoea

Laboratory Findings in Participants in  
Travelers' Diarrhea Study

	<u>Category I</u>	<u>Category II</u>		<u>Total</u>
	<u>Cases</u>	<u>Loose Stool Only</u>	<u>Well</u>	
Toxigenic <u>E. coli</u>	19	3	3	25
Salmonella <sup>*</sup>	4	1	4	9
<u>Shigella flexneri</u>	1			1
<u>Entamoeba histolytica</u>			1	1
<u>Giardia lamblia</u>			1	1
Reovirus <sup>**</sup>	1			1
Multiple pathogens <sup>***</sup>	7			7
No pathogen isolated	19	9	34	62
TOTAL	51	13	43	107

# Classes of diarrhoeagenic E. coli

1. Enterotoxigenic
2. Enteroinvasive
3. Enteropathogenic
4. Enterohemorrhagic
5. Enteroaggregative
6. Diffuse-adhering



**TABLE 78-4 ■ Clinical and Pathologic Features of Diarrheagenic *E. coli* Infection**

<i>E. COLI</i> STRAIN	CLINICAL SYNDROME	SITE OF INFECTION	HISTOPATHOLOGY
ETEC	Watery diarrhea	Small bowel	None
EPEC	Watery diarrhea	Small bowel	Effacement of brush border
EAEC	Watery diarrhea	Small bowel	Not well- described
EIEC	Dysentery	Large bowel	Invasive, inflammatory
EHEC	Hemorrhagic colitis, hemolytic- uremic syndrome	Large bowel	Effacement of brush border, not inflammatory

## APPENDIX 20-2. EXOTOXINS PRODUCED BY ENTEROBACTERIACEAE

Toxin	Target	Enzymatic Activity	Primary Action	Effect	Genetic Control
<i>Escherichia coli</i> LT	G regulatory protein	ADP-ribosylation	Adenylate cyclase stimulation	Fluid loss	Plasmid
<i>Escherichia coli</i> ST	Glycoprotein receptor	Unknown	Guanylate cyclase stimulation	Fluid loss	Plasmid
<i>Shigella dysenteriae</i> , type 1 Shiga toxin	60S ribosome	Modifies 28S ribosomal RNA	Inhibits protein synthesis	Cell death	Chromosomal
Shigalike toxins	60S ribosome	Modifies 28S ribosomal RNA	Inhibits protein synthesis	Cell death	Temperate phage



Mouse Injected Intra-gastrically with Normal E. coli strain



Mouse Injected Intra-gastrically with E.coli that Produces the ST enterotoxin

**TABLE 4.** Etiologies of Travelers' Diarrhea

<i>Characteristics</i>	<i>Latin America (15 Studies)</i>	<i>Africa (3 Studies)</i>	<i>Asia (8 Studies)</i>
Duration of stay (days)	21 (2–42) <sup>a</sup>	28 (28–35)	(28–42)
Attack rate (%)	52 (21–100)	54 (36–62)	(39–57)
Percentage with			
Enterotoxigenic <i>E. coli</i>	46 (28–72)	36 (31–75)	(20–34)
<i>Shigella</i>	0 (0–30)	0 (0–15)	(4–7)
<i>Salmonella</i>		0 (0–0)	(11–15)
<i>C. jejuni</i>	—	—	(2–15)
<i>V. parahaemolyticus</i>	—	—	(1–13)
Rotavirus	23 (0–36)	0 (0–0)	—

<sup>a</sup> Median (range) from 26 studies.<sup>258,259</sup>

# Tips to Avoid Traveler's Diarrhea

## Safe and unsafe foods in developing tropical regions

### Low-risk foods and beverages

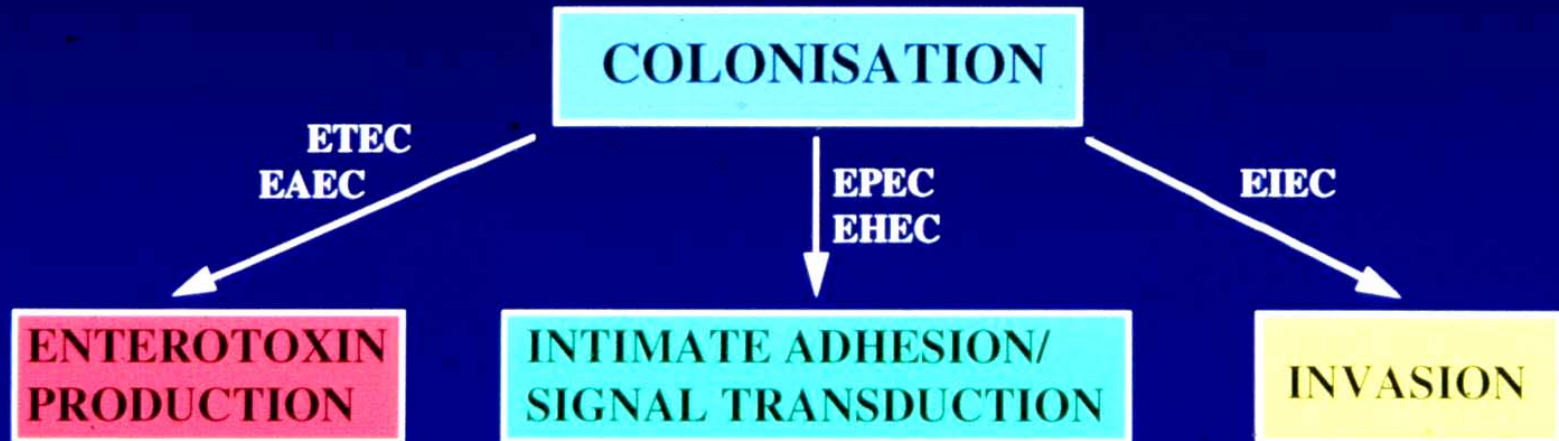
Any item served steaming hot (> 59° C)  
Foods that are dry (*ie*, bread and crackers)  
Items with very high sugar content  
(syrups and jellies)  
Fruits and vegetables that have been peeled  
Peanut butter  
Any fresh food item properly washed and prepared  
by the traveler  
Bottled carbonated drinks including mineral water,  
soft drinks, and beer

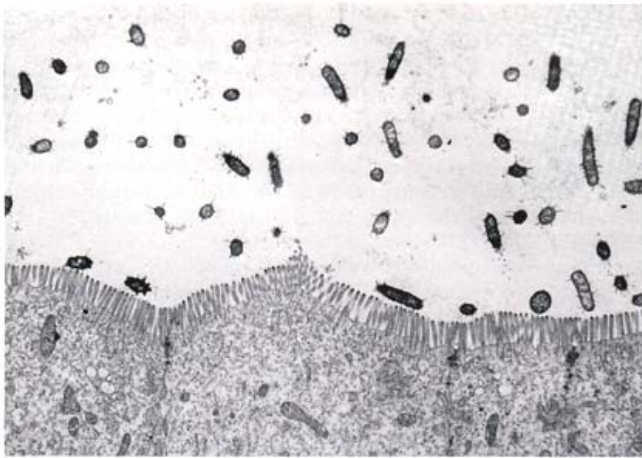
### High-risk foods and beverages

Foods that are moist and served at room temperature,  
especially those at a buffet  
Fruits and vegetables with skin intact—strawberries,  
tomatoes, grapes  
Salads and other uncooked vegetables  
Sauces and dressings in open containers on the table  
Milk (other than powdered milk that is constituted with  
previously boiled water or irradiated milk kept refrigerated  
after preparing or opening)  
Tap water or ice

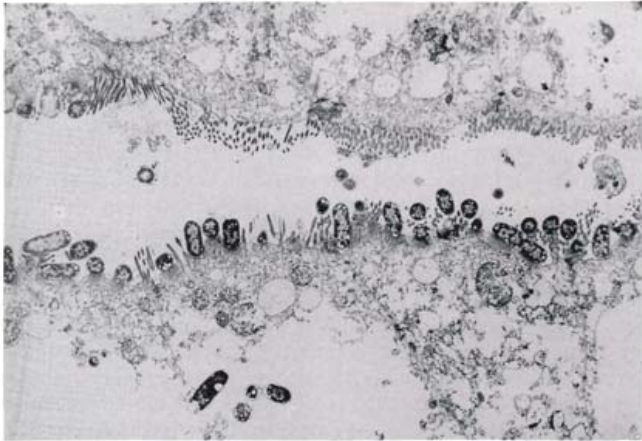
# Common Themes in *E. coli* Virulence

- (i) colonisation of a mucosal site
- (ii) evasion of host defenses
- (iii) multiplication
- (iv) host damage





ETEC does not adhere tightly  
Columnar gut epithelial cells intact  
But host had severe diarrhea → from toxins



EPEC causes attaching and effacing lesions



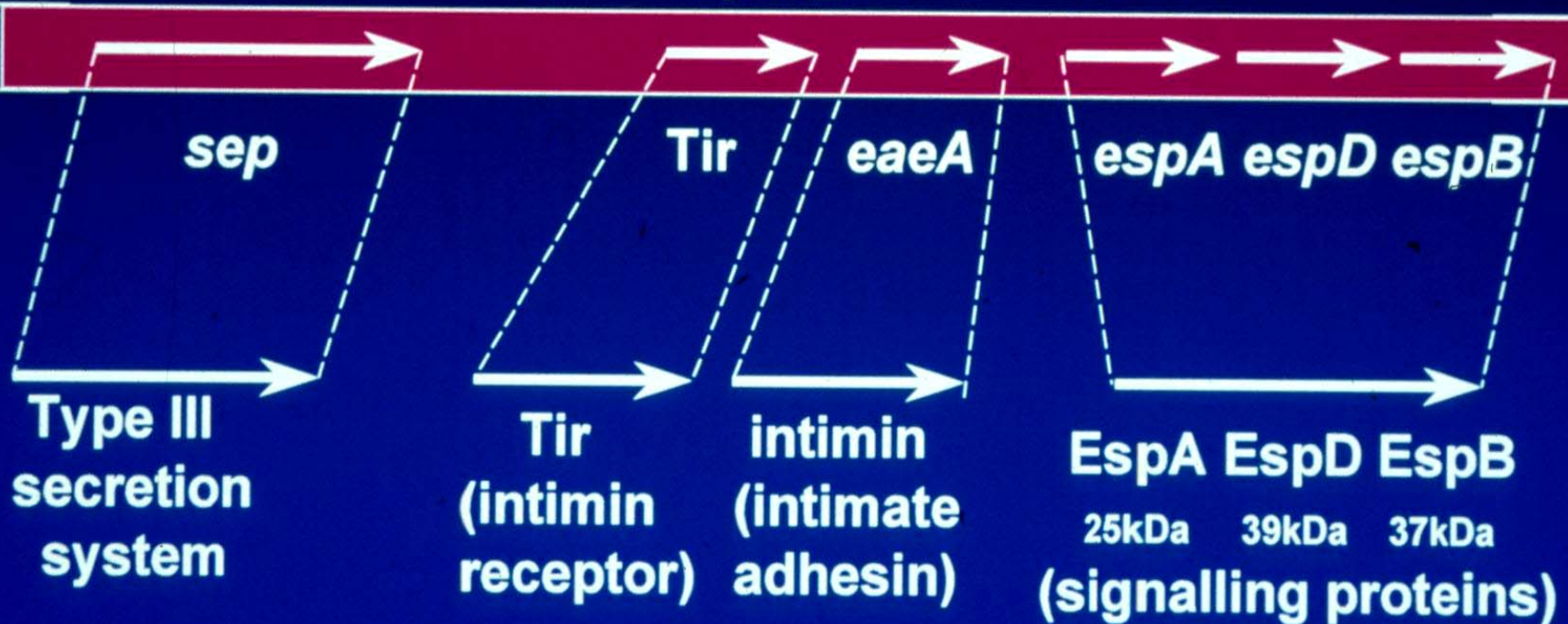
Intimate  
attachment



Pedestal  
formation

# Enteropathogenic Escherichia coli

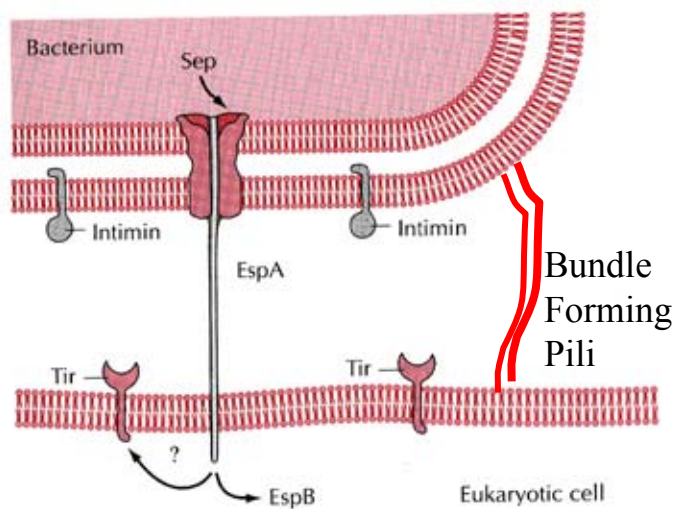
35kb locus of enterocyte effacement - LEE

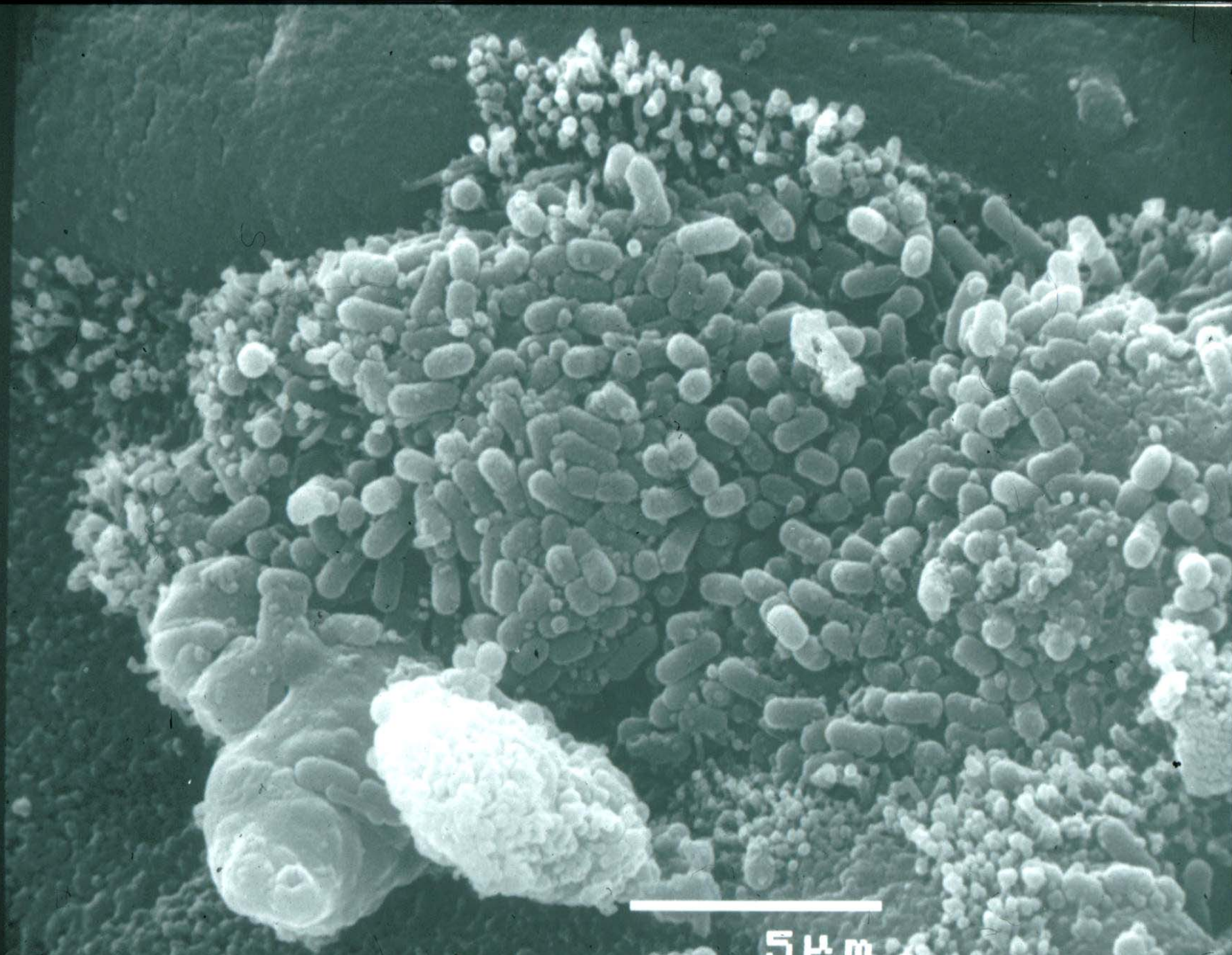


# EPEC: induction of tight adherence and pedestal formation

## 3-Stage Process:

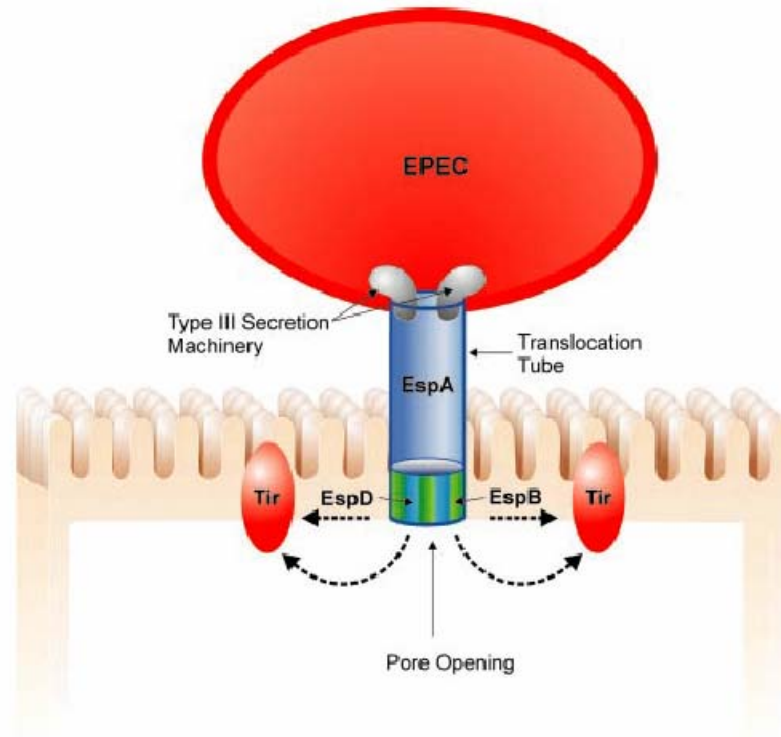
Step 1: initial adherence  
formation of microcolonies





# EPEC: induction of tight adherence and pedestal formation

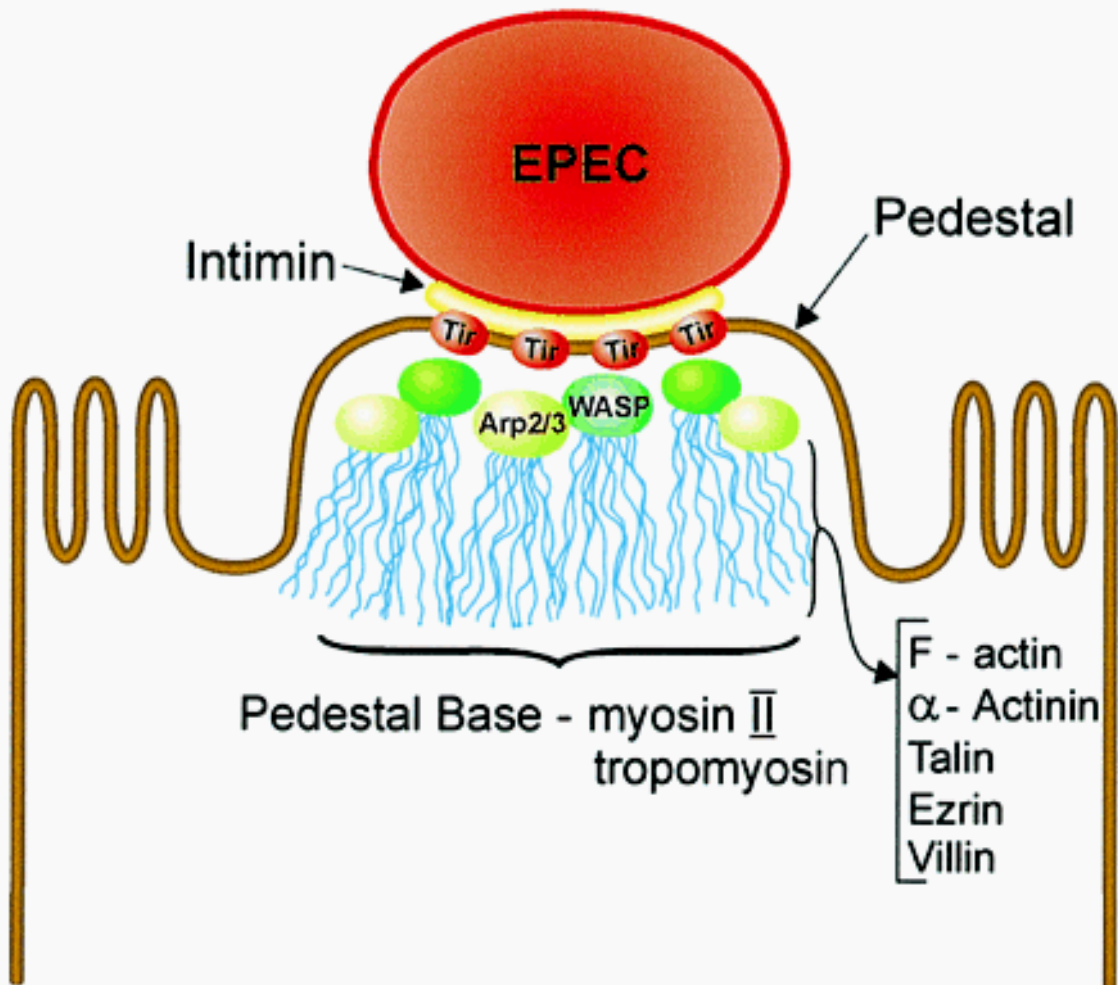
## Step 2: translocation of bacterial proteins



**Fig. 1.** Translocation of EPEC-secreted proteins (EspA) occurs through a type III secretion system that forms a pore through EPEC's membranes. Once translocated outside the bacteria, EspA forms a filamentous translocation tube whereas EspB and EspD are inserted into the host cell membrane, putatively forming a pore structure, allowing the passage of other effector proteins, such as Tir into the host cell membrane.

# EPEC: induction of tight adherence and pedestal formation

## Step 3: intimate adherence enterocyte effacement pedestal formation

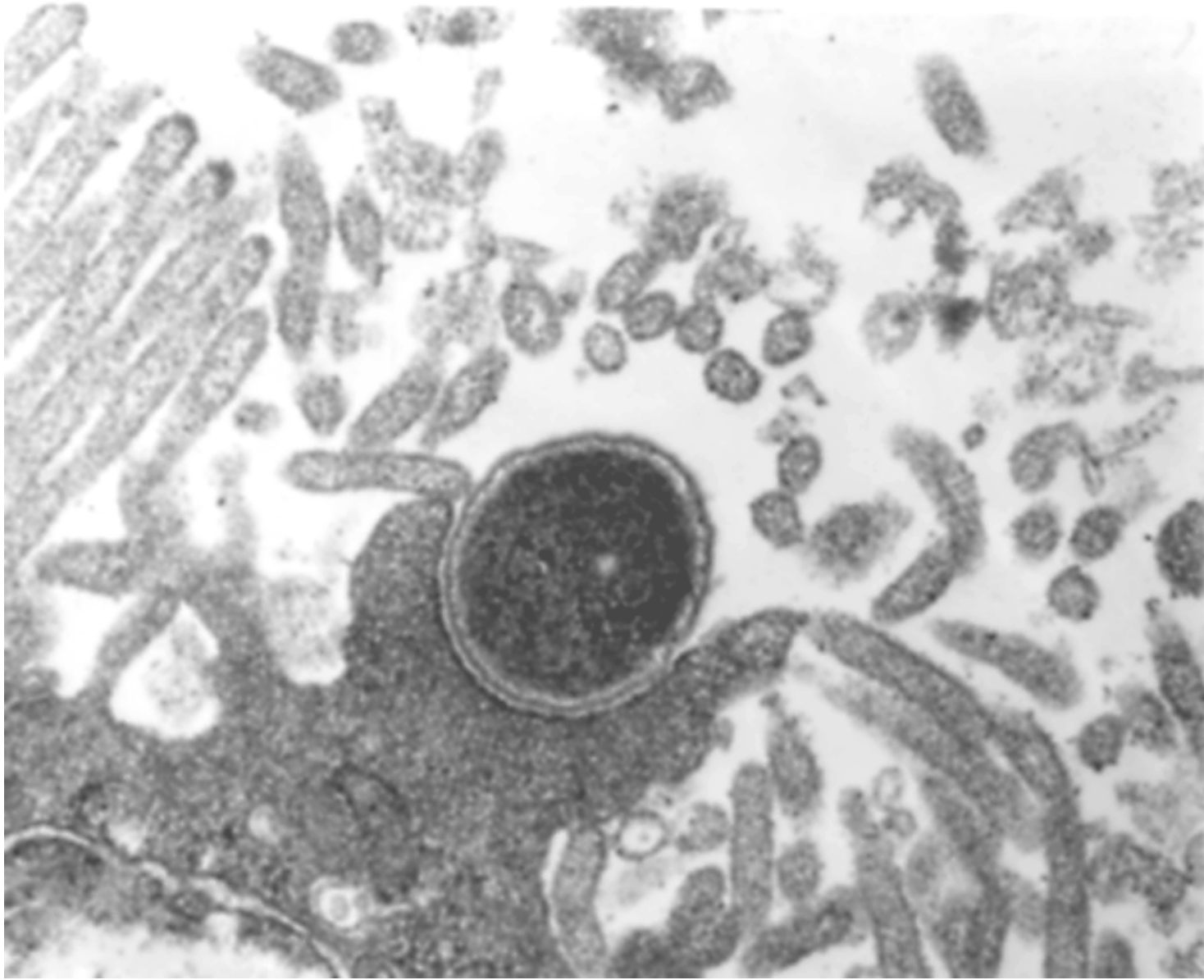


### Intimin (in bacterial membrane):

- 9 kDa outer membrane protein
- adhesin
- C-term 280 aa binding domain

### Tir (in host cell membrane):

- 78 kDa → 90 kDa in host
- translocated into host cell memb.
- Tyrosine phosphorylated
- binds to intimin
- Role in pedestal formation and actin condensation

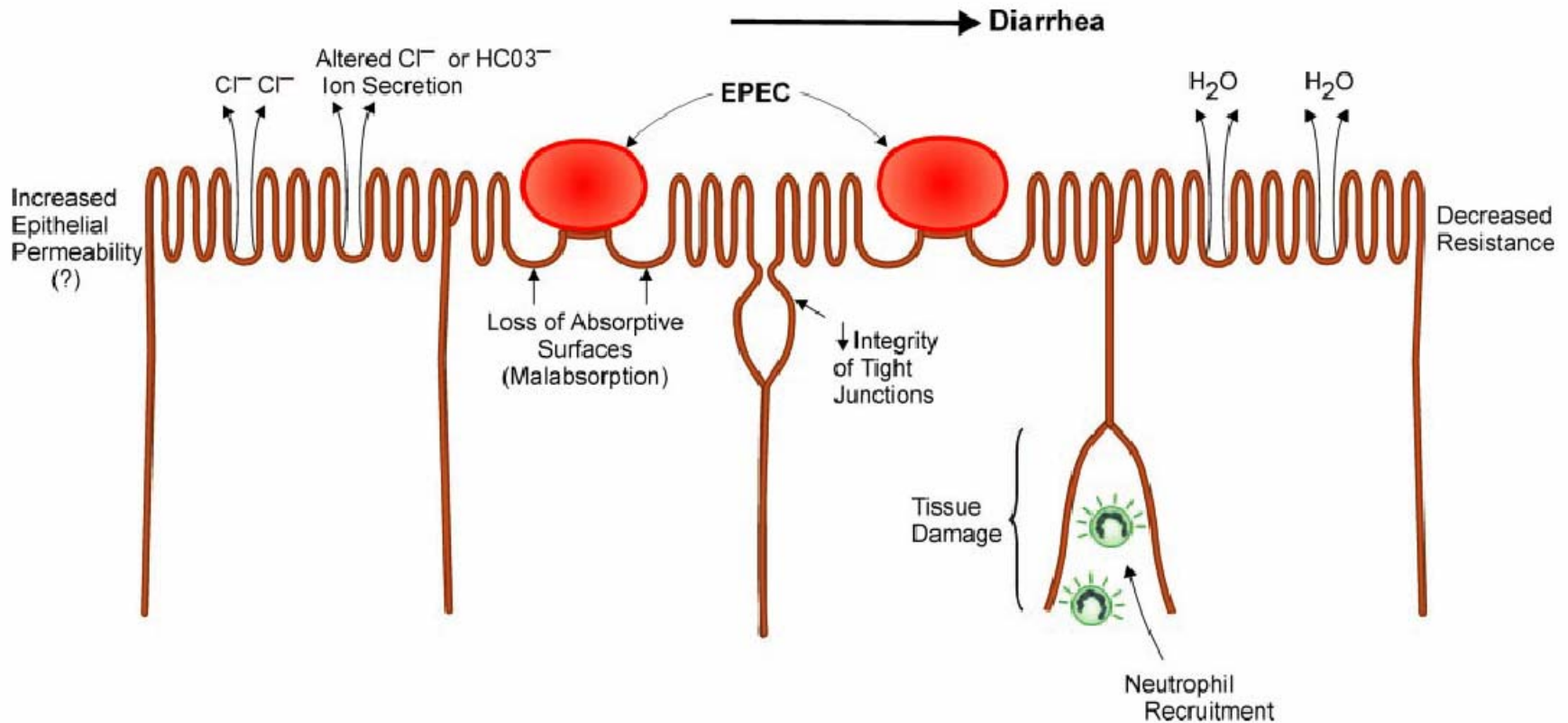


The Attachment and Effacing Lesion of Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC)

# EPEC pedestals



# How does EPEC cause diarrhea?



**Fig. 5.** Putative mechanisms underlying EPEC induced diarrhea include increased epithelial permeability and alterations in  $\text{Cl}^-$  and  $\text{HCO}_3^-$  ion secretion. Contributing structural changes include loss of absorptive surfaces, reduced tight junction integrity, and tissue damage.

# Enterohemorrhagic *E. coli*



*E. coli* O157

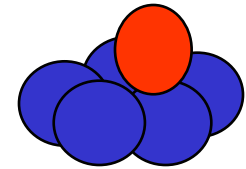
- EHEC are usually transmitted to humans through contaminated food or drink.
- Generally speaking EHEC is carried asymptotomatically in a number of animals, bovine species, particularly.

# Shiga toxin

Produced by *Shigella dysenteriae* and some *E.coli* species like O157

- enterotoxin activity (fluid release)
- paralysis when injected into animals
- cytotoxic to cell cultures

In addition to endothelial damage in bowel and haemorrhage it can cause **Haemolytic Uremic Syndrome** - as a complication..

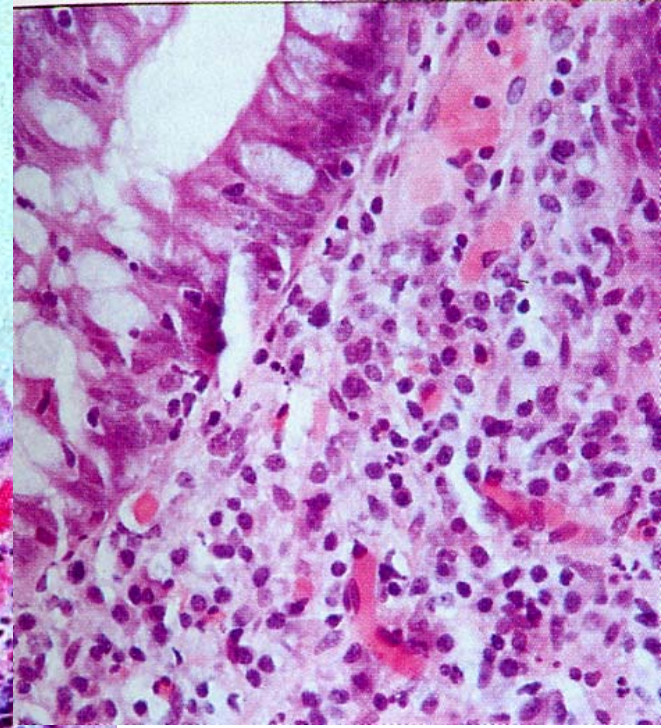


**B** subunit binds to glycolipid Gb3 or Gb4 (distinct from GM1)

**A** subunit contains N-glycosidase activity

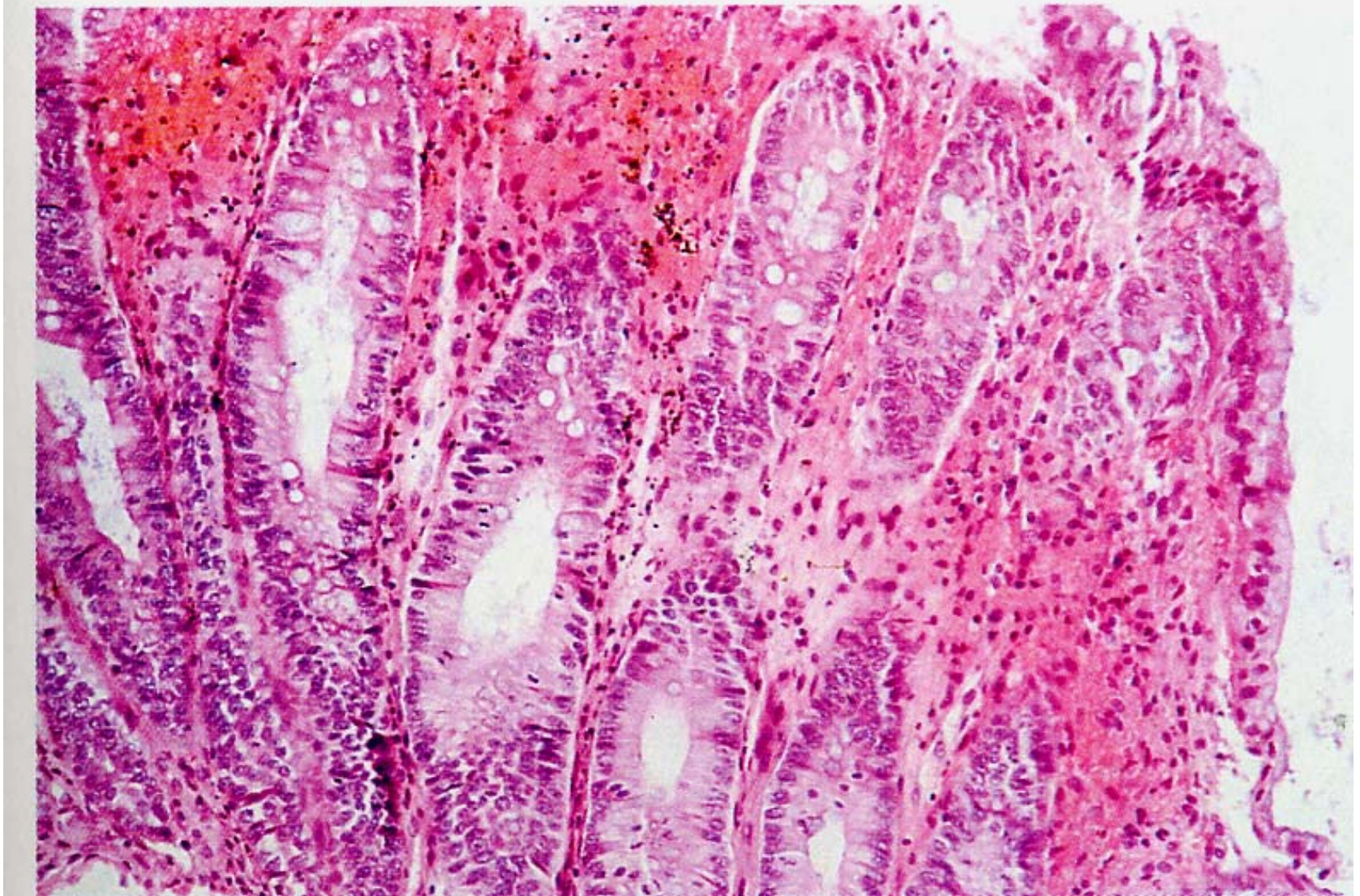
- Intracellular target - adenosine 4324 within 28S rRNA of 60S ribosomal subunit
- Cleavage of N - glycosidic bond prevents binding of amino-acyl tRNAs
- Inhibition of eukaryotic protein synthesis
- No effect on bacterial ribosomes
- same mechanism of action as ricin - potent plant toxin

Toxin	Disease	% homology with ShT	Glycolipid receptor
ShT	Bacillary dysentery	100%	Gb3
SLT-1	HUS	99%	Gb3
SLT-11	HUS	58%	Gb3
SLT-11v	Swine dysentery	80%	Gb4

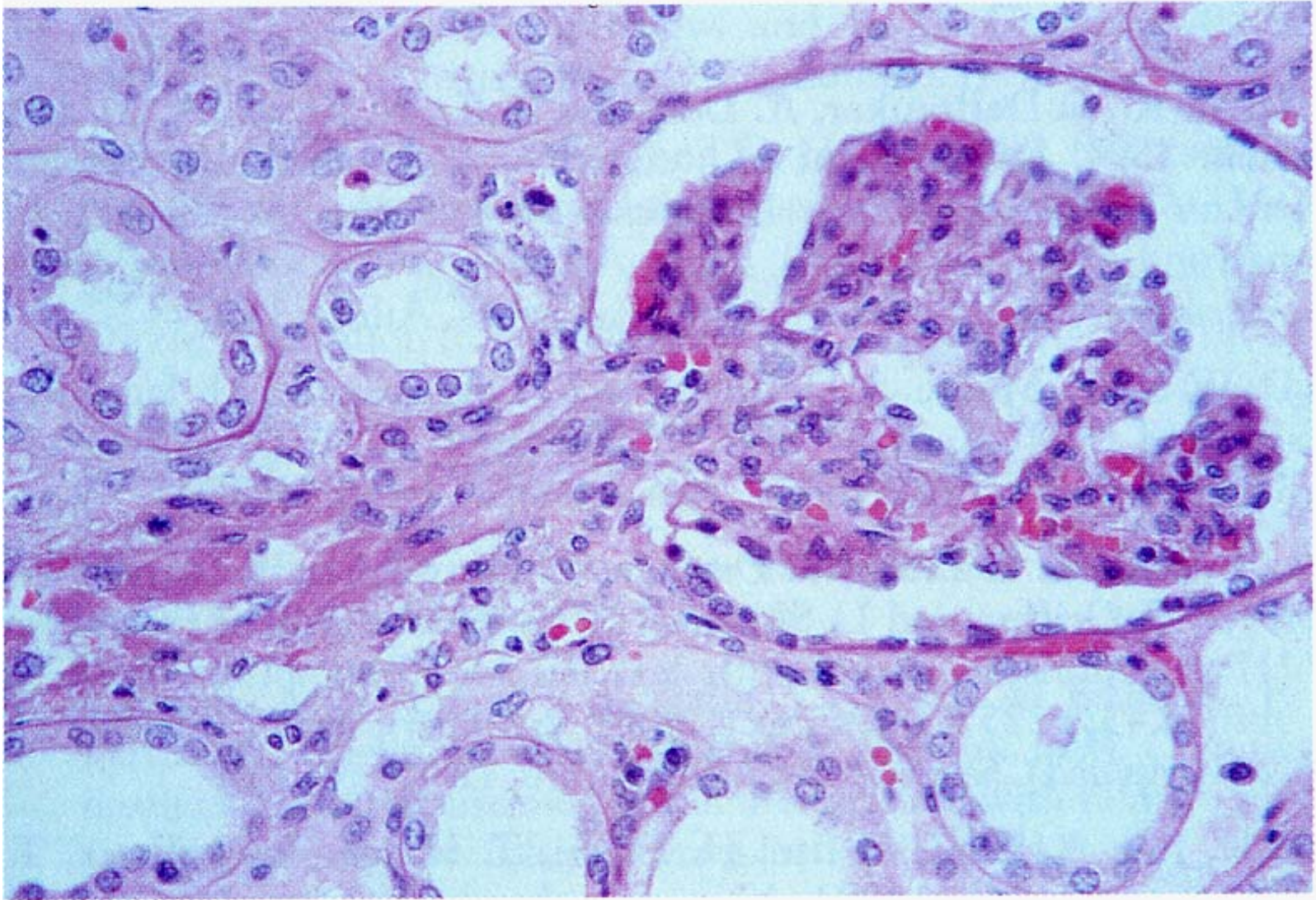


E. Coli O157 colitis: colonic mucosa shows hemorrhage and edema of lamina propria.

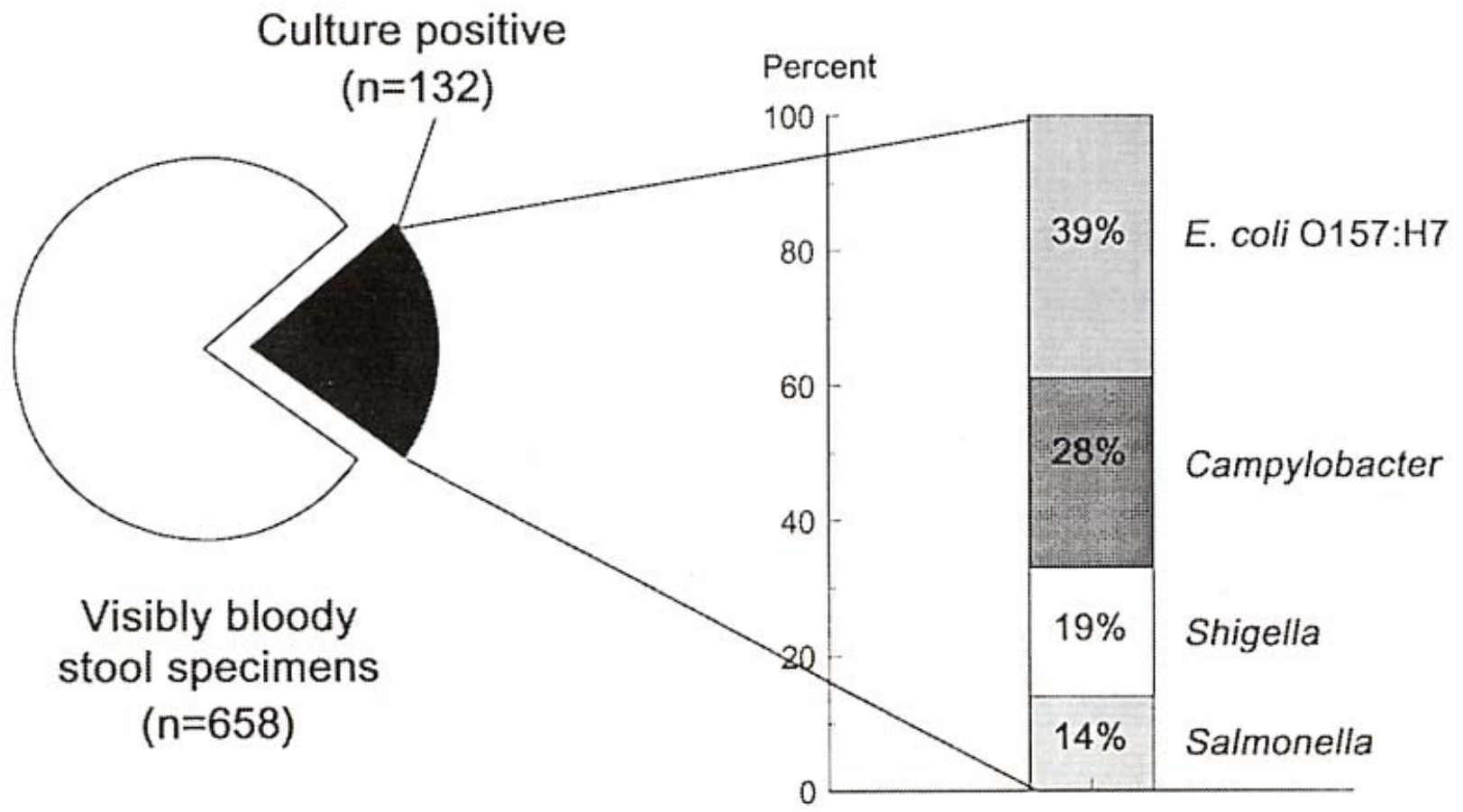
In this higher magnification, the lamina is seen to contain neutrophils and mononuclear inflammatory cells



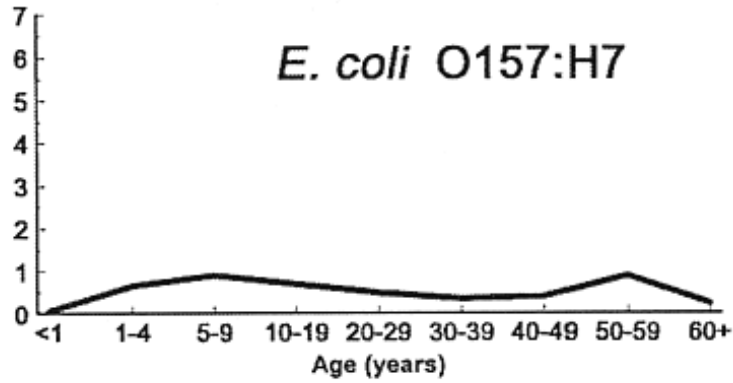
Ischemic pattern of *E. coli* O157 colitis. The colonic mucosa shows hemorrhage of the lamina propria with partial coagulation and necrosis of the surface epithelium. The deeper colonic crypts are preserved.



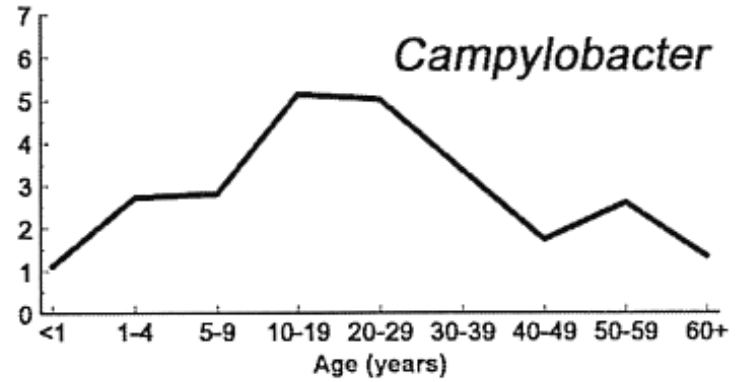
Hemolytic Uremic syndrome following *E. coli* O157 infection. The glomerulus shows a fibrin thrombus in the afferent arteriole and necrotic glomerular tufts



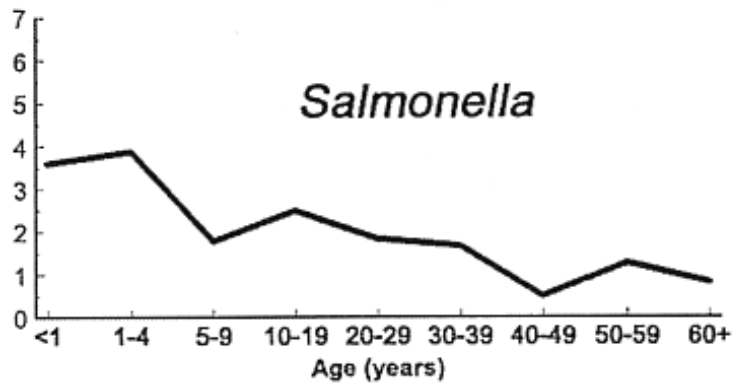
Percent of positive fecal specimens



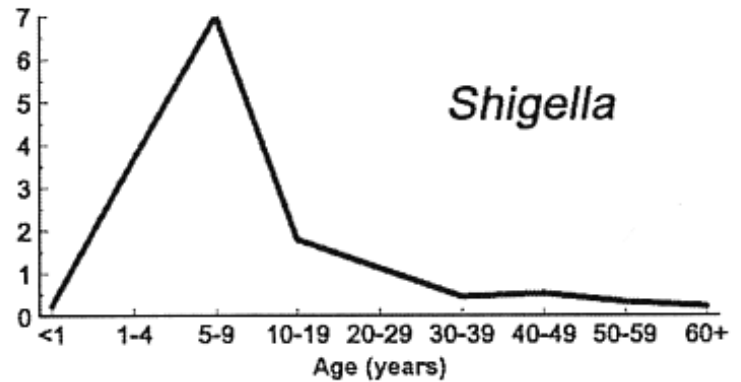
Percent of positive fecal specimens



Percent of positive fecal specimens



Percent of positive fecal specimens



N=30,463 fecal specimens

- Break

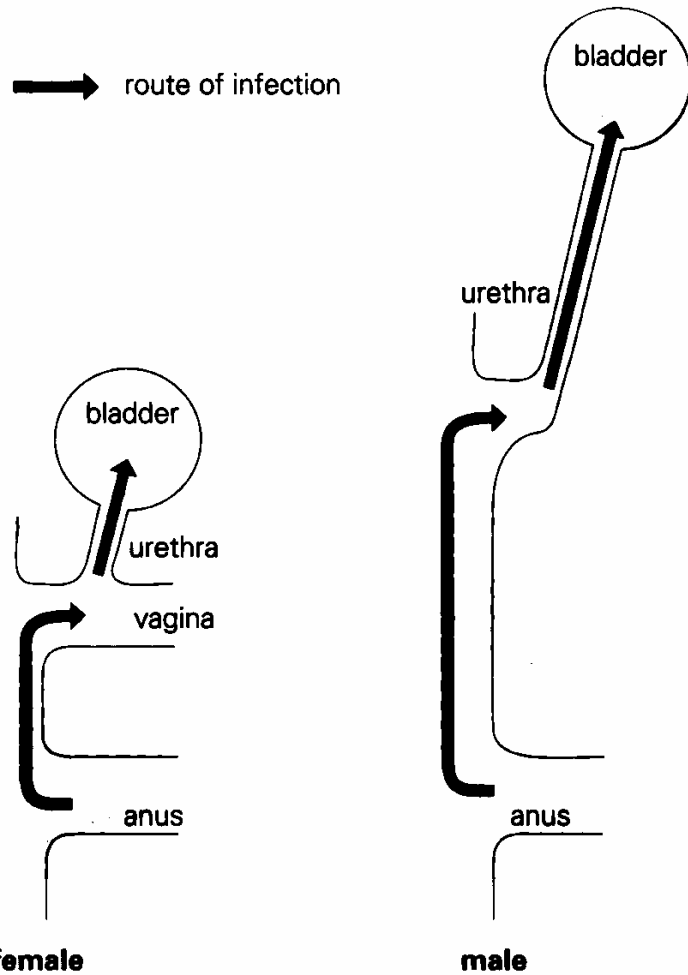
# *E. coli* is the Most Common Cause of Urinary Tract Infection

TABLE 112-1 ■ Microbial Species Most Often Associated With Specific Types of UTIs

MICROBE	ACUTE UNCOMPLICATED CYSTITIS (%)	ACUTE UNCOMPLICATED PYELONEPHRITIS (%)	COMPLICATED UTI (%)	CATHETER-ASSOCIATED UTI (%)
<i>E. coli</i>	79	89	32	24
<i>S. saprophyticus</i>	11	0	1	0
<i>Proteus</i>	2	4	4	6
<i>Klebsiella</i>	3	4	5	8
Enterococci	2	0	22	7
<i>Pseudomonas</i>	0	0	20	9
Mixed	3	5	10	11
Other	0	2	5	10
Yeast	0	0	1	28
<i>S. epidermidis</i>	0	0	15	8

(Data in columns one and two are from 607 episodes of cystitis and 84 episodes of pyelonephritis in Seattle; data from columns 3 and 4 from Platt R, et al: Am J Epidemiol 124:977, 1986 and Gasser TC, et al: Am J Med 82(Suppl 4):278, 1987.)

## VULNERABILITY OF FEMALE UROGENITAL TRACT



□ 1/3 of American women  
Will have at least one  
UTI before age 65

Many will experience  
One per year

Reservoirs

large intestine

vagina

periurethral tissue

**Fig. 11.11** The female urinogenital tract is particularly vulnerable to infection, due mainly to topographical considerations.

## Epidemiology of bacterial cystitis

---

20%–30% of women experience an episode of cystitis during their lifetime

~20% of women experience recurrent infections

Recurrent infections are often clustered: two thirds occur within 6 months

Annually, in the United States, bacterial cystitis accounts for:

7 million visits to physicians' offices

1% of clinic visits

\$1 billion in outpatient care costs

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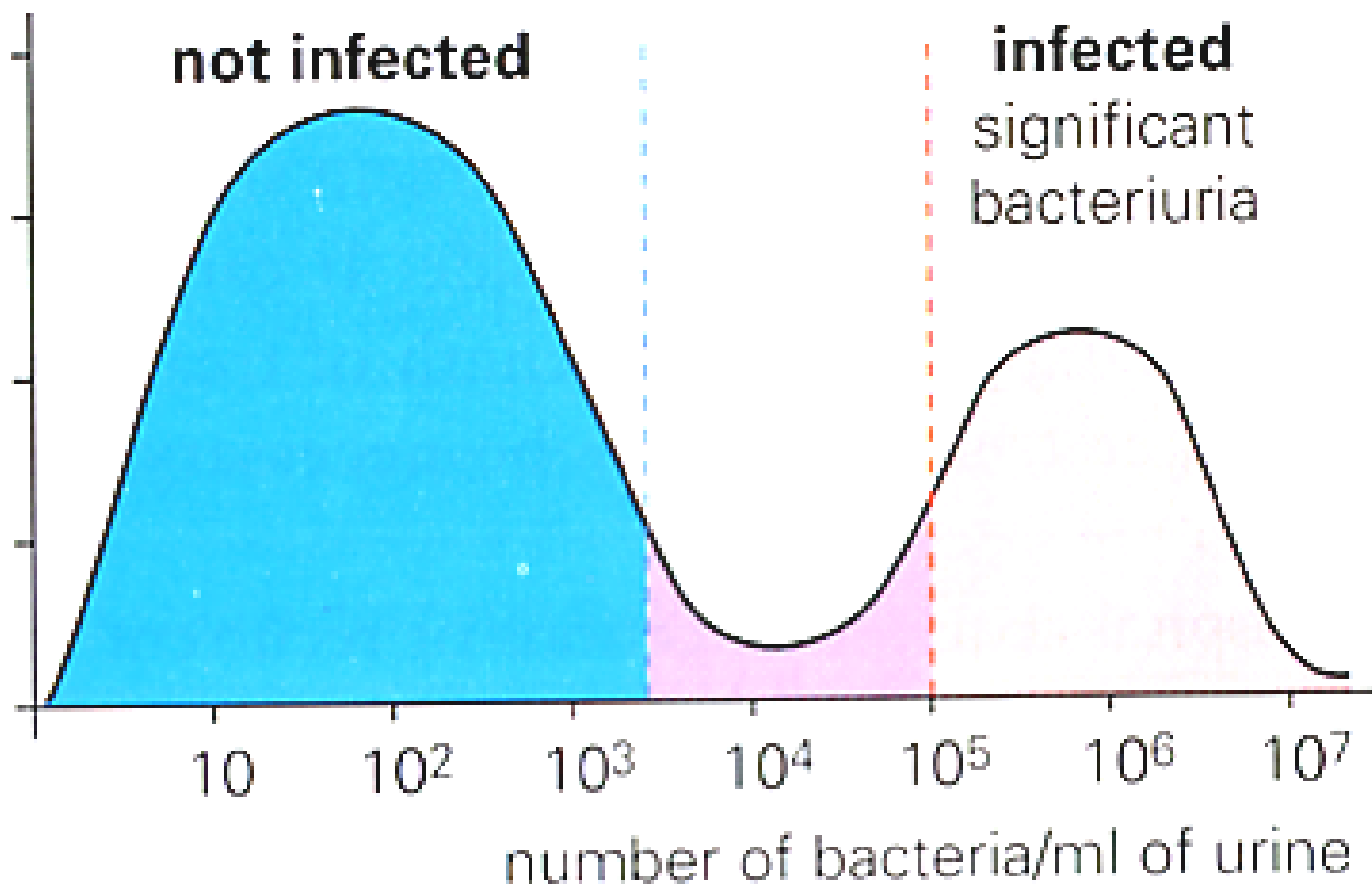
**TABLE 112-2 ■ Overview of the Epidemiology of UTI by Age Group**

AGE GROUP (yr)	FEMALES		MALES	
	Prevalence (%)	Risk Factors	Prevalence (%)	Risk Factors
<1	1	Anatomic or functional urologic abnormalities	1	Anatomic or functional urologic abnormalities
1-5	4-5	Congenital abnormalities, vesicoureteral reflex	0.5	Congenital abnormalities, uncircumcised penis
6-15	4-5	Vesicoureteral reflux	0.5	None
16-35	20	Sexual intercourse, diaphragm use	0.5	Homosexuality
36-65	35	Gynecologic surgery, bladder prolapse	20	Prostatic hypertrophy, obstruction, catheterization, surgery
Over 65	40	As above, plus incontinence, chronic catheterization	35	As above, plus incontinence, long-term catheterization

number of patients

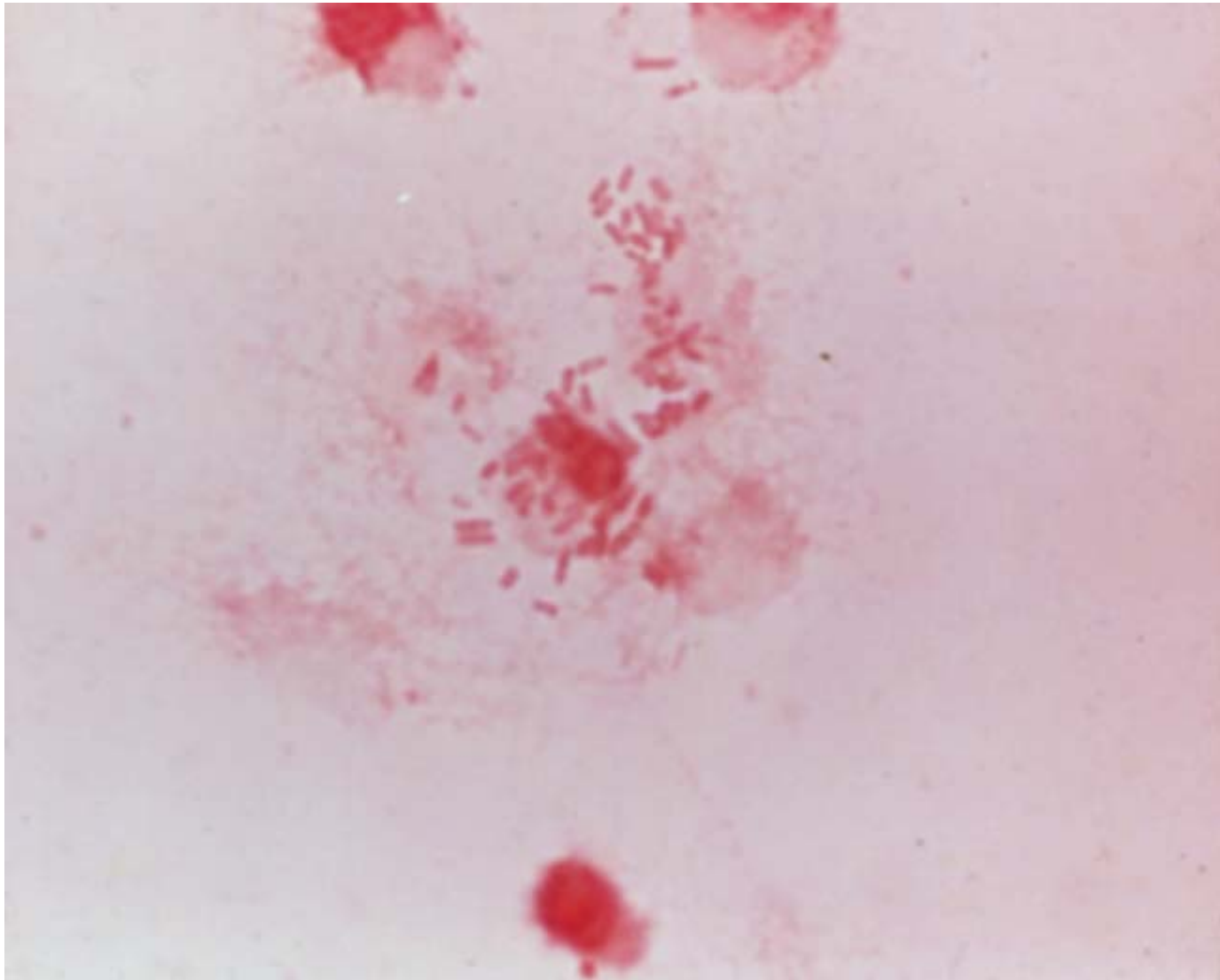
**not infected**

**infected**  
significant  
bacteriuria



--- not delivered promptly  
or badly collected

--- probable infections but  
require confirmation



Gram Stain of Unspun Urine from a Case of Acute Cystitis.

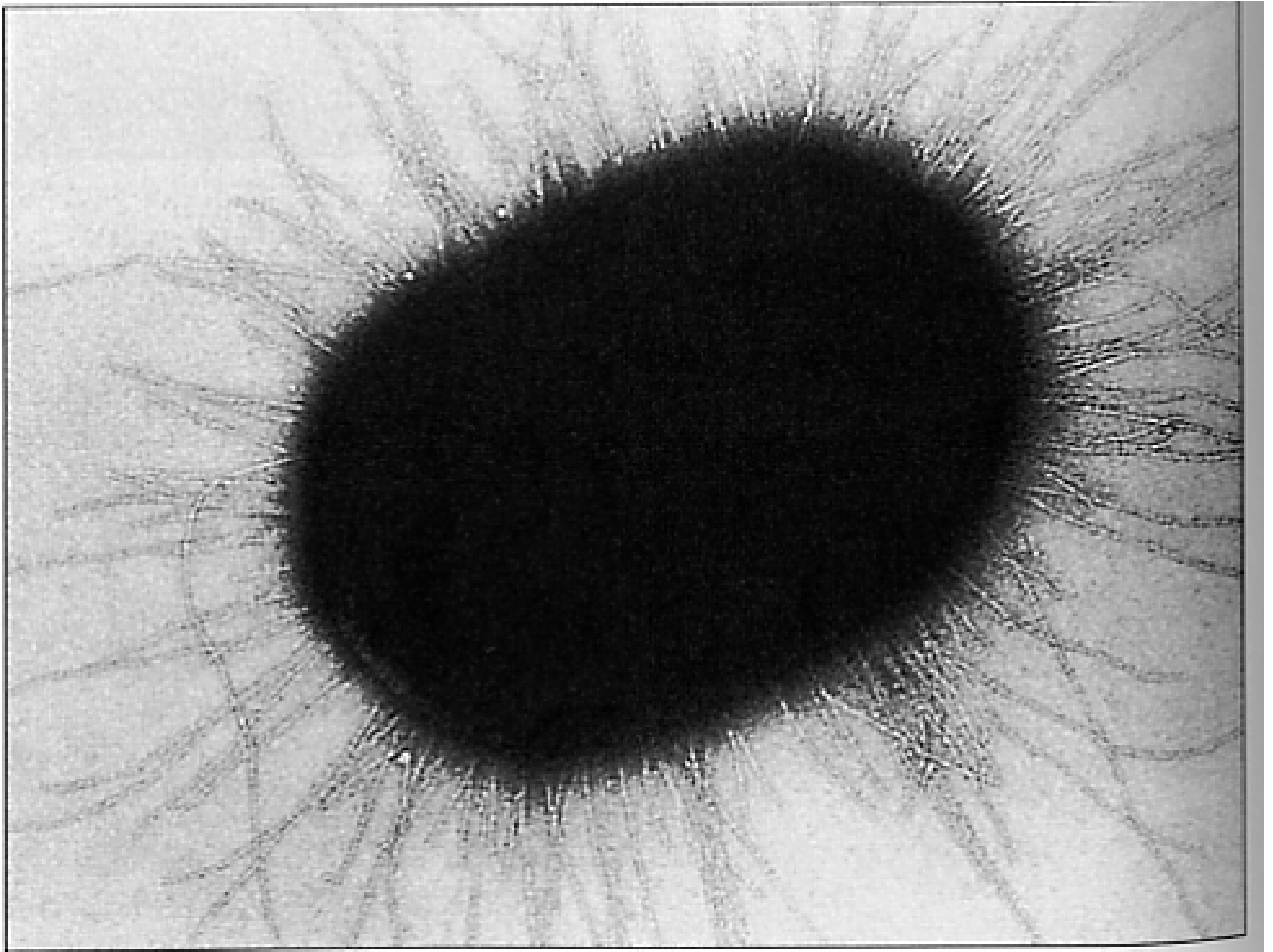
# The Genetic Basis for Uropathogenicity

Once bacteria have gained access to the bladder, they must overcome the host defense mechanisms

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- 1) Flushing action of urine
- 2) Tamm-Horsfall protein
- 3) Uromucoid
- 4) Secretory IgA

Therefore uropathogenic organisms must adhere to urinary epithelial cells.



## Pathogenic E. coli make a number of adhesins

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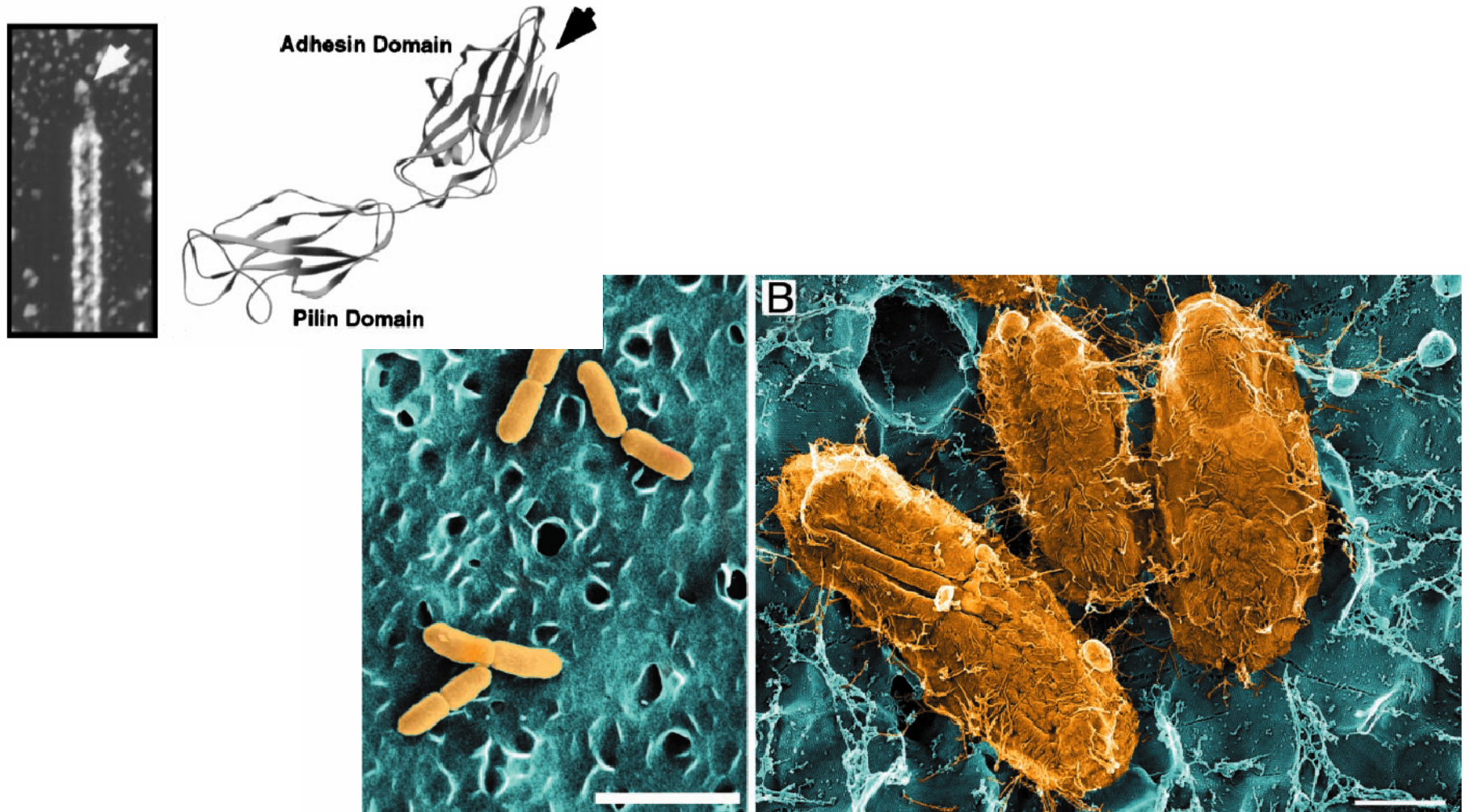
**P pili** - in strains that cause kidney infections  
bind globobiose ( $\alpha$ -D-Gal-(1,4)- $\alpha$ -D-Gal)

**Dr adhesins** - binds the Dr blood group

**S pili** - in strains that cause neonatal meningitis

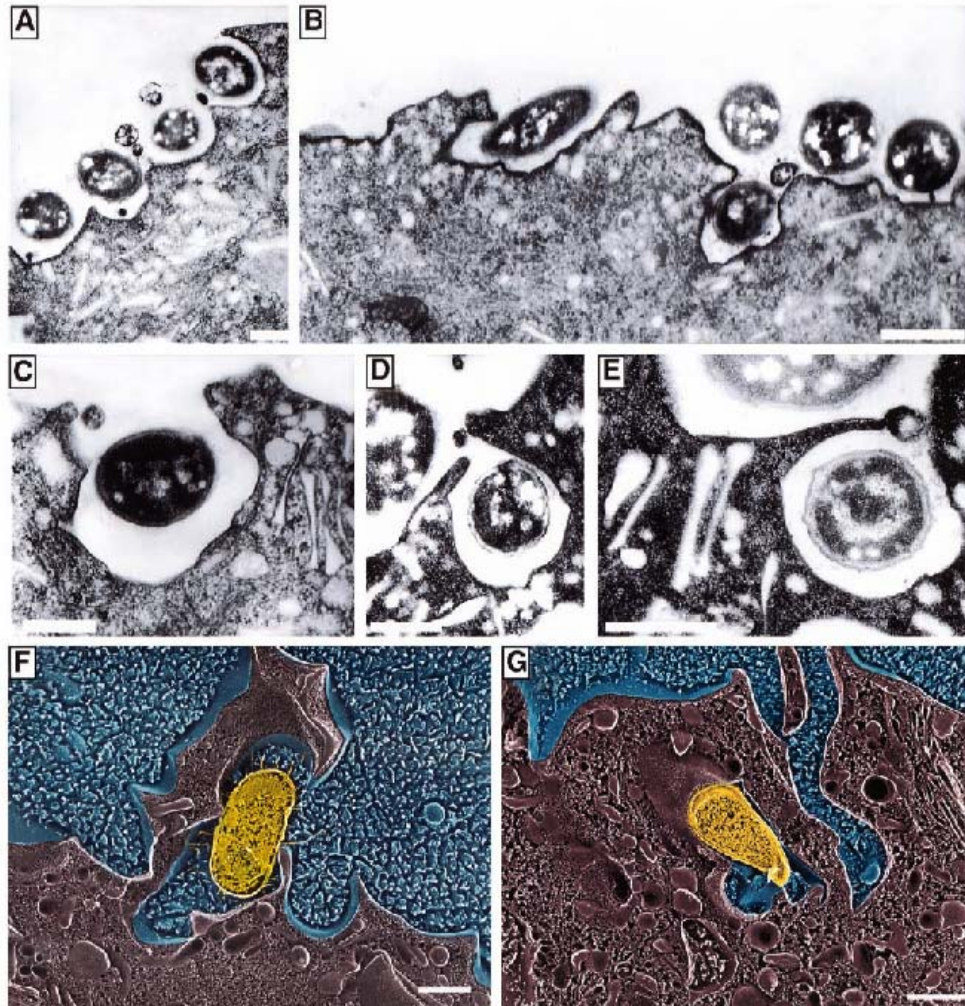
**Type I pili** - assoc. w/ ability to colonize urinary tract  
bind mannose residues on bladder  
glycoproteins (uroplakins)

# Type I pilus mediates bacterial attachment to the bladder epithelium



**Fig. 2.** Type 1 pilus-mediated bacterial attachment to the bladder epithelium. After inoculation of C57BL/6 mice with type 1-piliated UPEC, numerous bacteria (yellow) can be found attached to the luminal surface of the bladder (blue) as detected by scanning EM (A) and high-resolution freeze-dry/deep-etch EM (B). Type 1 pili mediating bacterial attachment were resolved with the high-resolution technique. The scalloped appearance of the bladder surface is attributable to the presence of the uroplakin plaques ( $\approx 0.5 \mu\text{m}$  in diameter). [Bars =  $3 \mu\text{m}$  (A) and  $0.5 \mu\text{m}$  (B).]

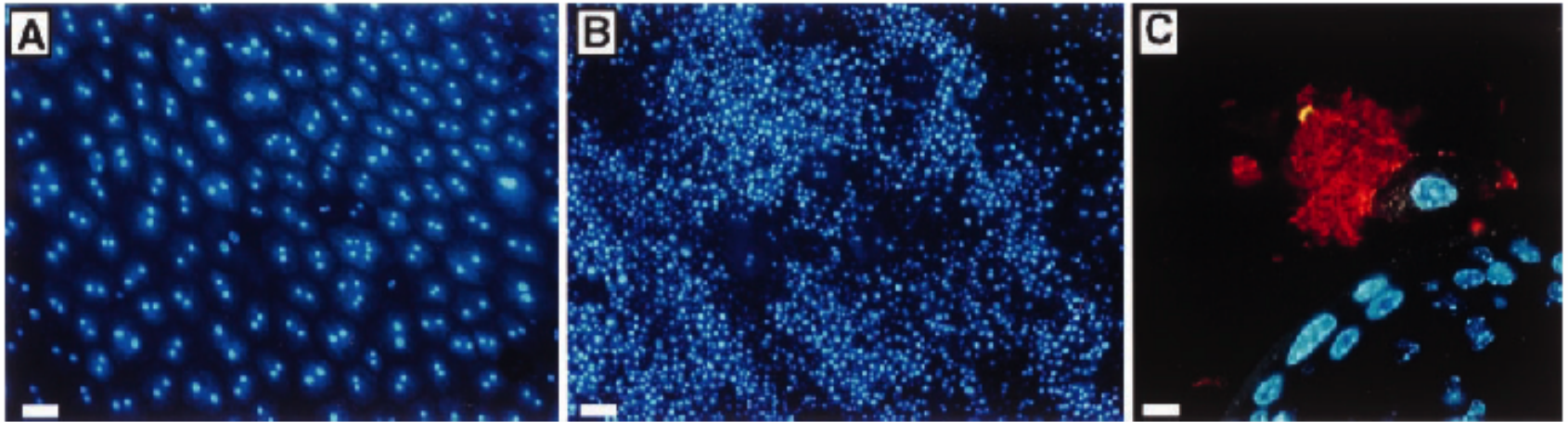
# Internalization of type 1-piliated UPEC by Superficial facet cells



Internalized *E. coli* have a distinct survival advantage over their external counterparts

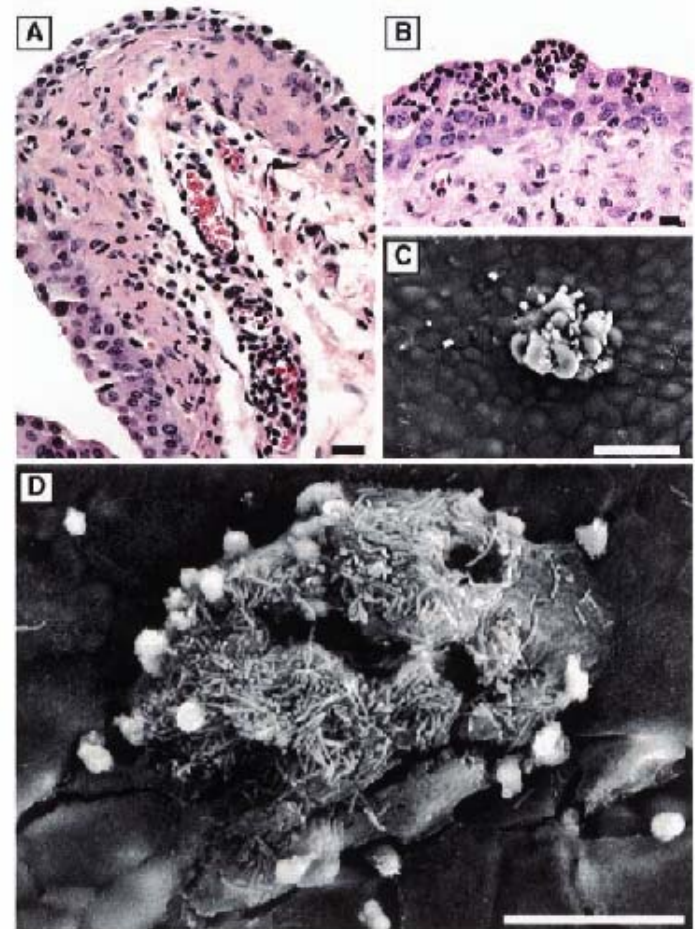
**Fig. 3.** Internalization of type 1-piliated UPEC by superficial facet cells. (A–F) Transmission EM shows the AUM of mouse superficial facet cells in various stages of enveloping adherent type 1-piliated UPEC. (F and G) High-resolution freeze-fracture/deep-etch EM also reveals type 1-piliated *E. coli* (yellow) seemingly being enveloped by facet cells (brown; the AUM and extracellular milieu are colored blue). The cytoplasmic face of three uroplakin plaques, separated by smooth interplaque regions, can be seen in the host membrane that is partially enveloping the bacterium in G. All images were obtained by using C57BL/6 mouse bladders recovered about 1 h after infection with UPEC. [Bars = 1  $\mu\text{m}$  (A and B) and 0.5  $\mu\text{m}$  (C–G).]

# Bladder Cell Exfoliation as a Host Defense



**Fig. 5.** Exfoliation of bladder epithelial cells. (A) The luminal surface of the bladder is normally covered by extremely long-lived, highly differentiated superficial facet cells with distinctive pentagonal or hexagonal outlines. These large cells (ranging from 20 to 150  $\mu\text{m}$  in diameter) are often bi- or multinucleate and can be easily identified on the surface of whole-mount bladders stained with Hoechst dye. (B) Within 6 h after inoculation with type 1-piliated *E. coli*, many infected facet cells in C57BL/6 mice exfoliate and are rinsed away, revealing the smaller, mono-nucleate underlying epithelial cells. (C) A stained paraffin section from an infected mouse bladder shows a facet cell in the process of exfoliating and taking a large mass of adherent *E. coli* with it. Host cell nuclei were stained with Hoechst dye whereas bacteria were stained red by using anti-*E. coli* primary and Cy3-labeled secondary antibodies. [Bars = 100  $\mu\text{m}$  (A and B) and 10  $\mu\text{m}$  (C).]

# Innate Immune response- Neutrophil Influx and Cytokines



**Fig. 6.** Neutrophil influx into the urothelium in response to infection. (A) Paraffin sections of C57BL/6 mouse bladders recovered 6 h after infection with type 1-piliated UPEC and stained with hematoxylin and eosin show PMNs (small, darkly stained cells) migrating from blood vessels within the lamina propria and into the urothelium. (B) PMNs appeared to aggregate beneath the luminal surface of the bladder and could occasionally be seen, by scanning EM, emerging in the vicinity of adherent bacteria on the surface of newly exposed immature urothelial cells (C). PMNs were also found associated with infected facet cells in the process of exfoliating (D). [Bars = 20  $\mu$ m (A), 10  $\mu$ m (B), 50  $\mu$ m (C), and 30  $\mu$ m (D).]

# Persistent and Recurrent UTIs

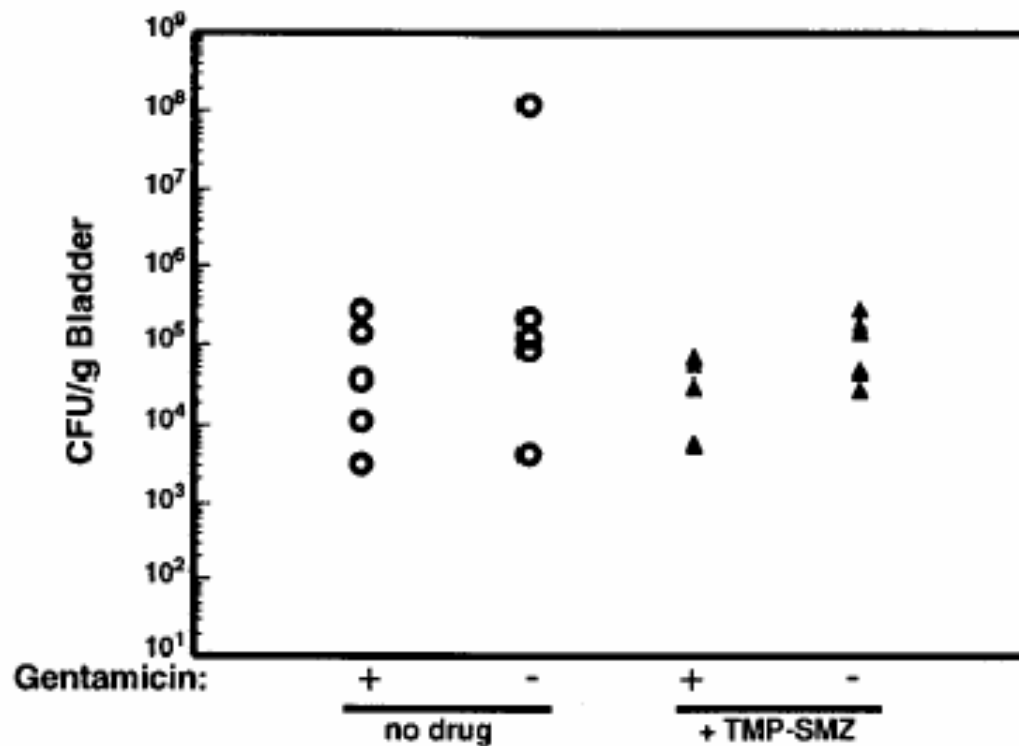
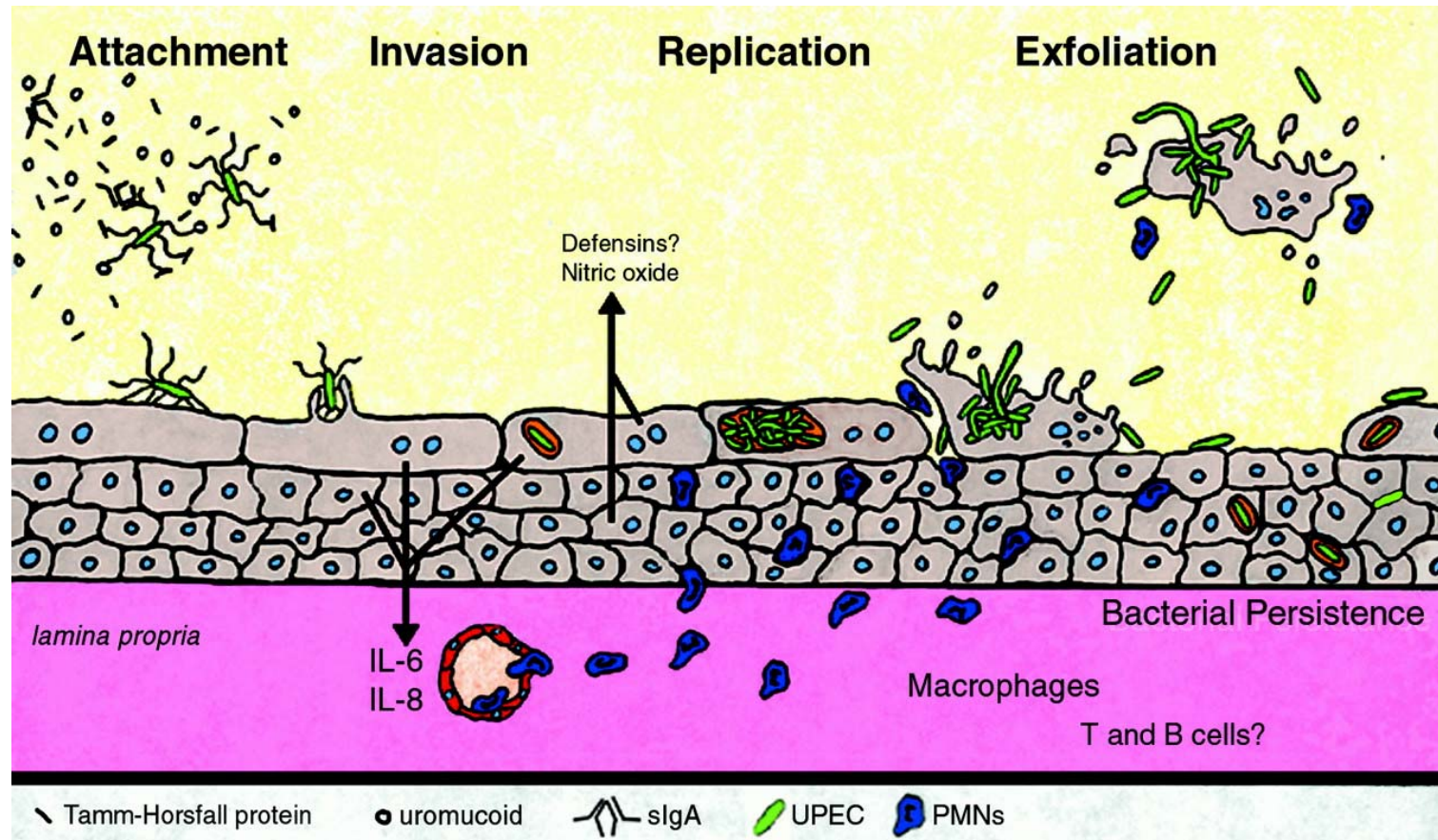


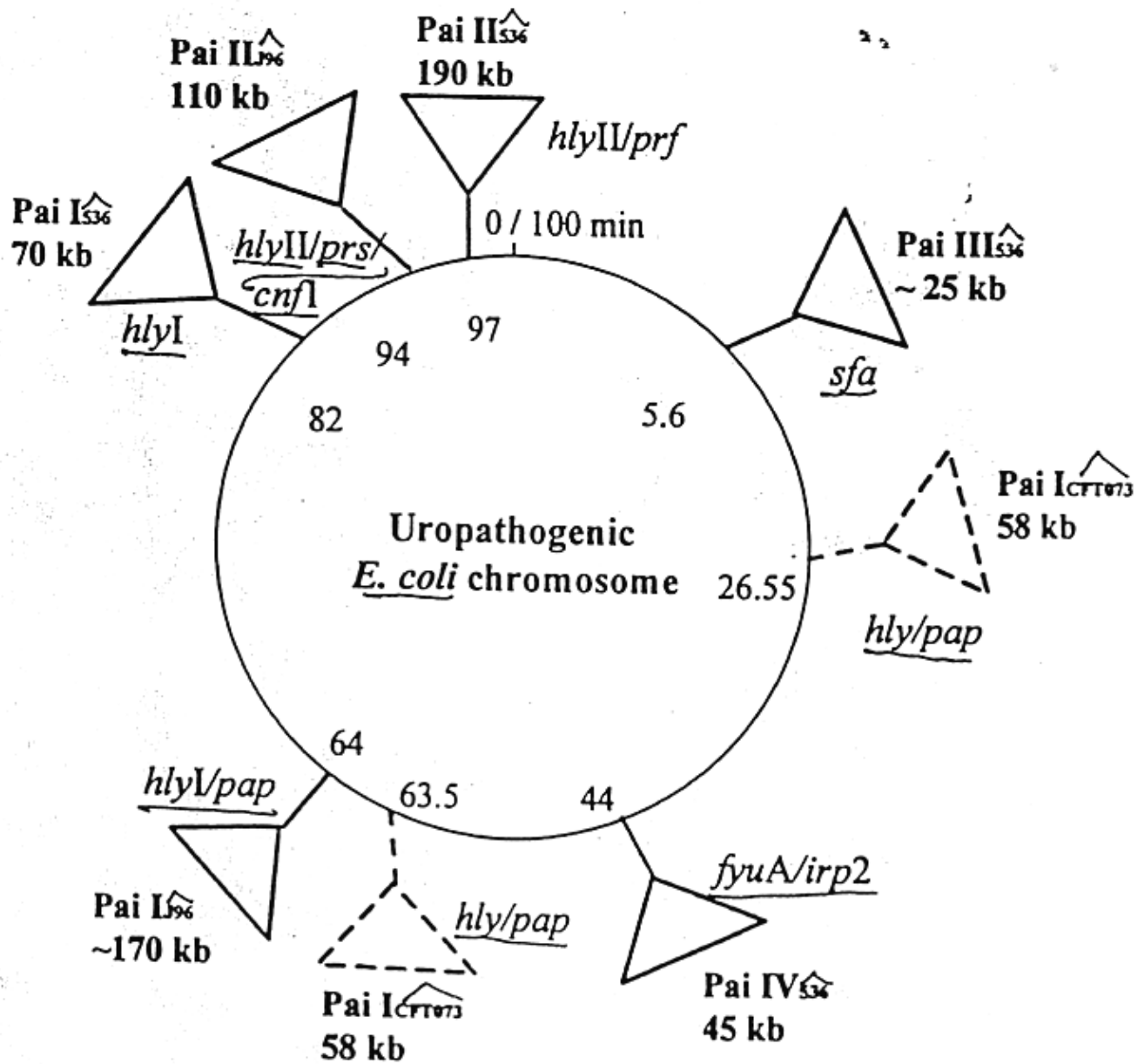
Fig. 7. Resistance of intracellular UPEC to antibiotic treatment. C57BL/6 mice were infected with  $1 \times 10^8$  colony-forming units of the clinical cystitis isolate NU14. Six hours after infection, mice were given trimethoprim-sulfamethoxazole (54  $\mu\text{g}/\text{ml}$  and 270  $\mu\text{g}/\text{ml}$ , respectively) in their drinking water or were left untreated. Mice were provided fresh water with or without antibiotics daily, and at 3 days after inoculation bladders were recovered and bisected. Bladder halves were treated with either gentamicin (to kill any extracellular bacteria) or were left untreated before homogenization and determination of bacterial titers. Control experiments (not shown) demonstrated that trimethoprim-sulfamethoxazole completely inhibited the growth of NU14 *in vitro*.

# Model of *E. coli* Cystitis



# Pathogenicity Islands

- often differ in GC content suggesting horizontal transfer
- often associated with tRNA genes
- usually carry cryptic or functional “mobility” genes such as integrases, transposases, IS elements
- present in pathogenic bacteria but absent from non-pathogenic members of same or closely related species
- may be flanked by directly repeated DNA sequences which could cause instability
- Large regions (10- >200Kb) regions containing genes encoding one or more virulence factors



# Shigella and Bacillary Dysentery

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## **What is shigellosis?**

Shigellosis is an illness caused by bacteria that affects the small and large intestine. It is transmitted when people eat food or drink water contaminated by shigella bacteria.

Illness usually begins 1 - 3 days after contact with the bacteria.

## **What are the symptoms?**

Symptoms usually include diarrhea, sometimes with blood or mucus, stomach cramps and fever, lasting from 4 to 7 days. Nausea and vomiting often occur.

The disease is more serious in infants, small children and the elderly.



# *Shigella*

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- **Lac<sup>-</sup> Enterobacteriaceae**
- non-motile
- no H<sub>2</sub>S formation
- no gas in glucose ferm.
- **Very closely related to E. coli.**
- **4 species based on O Ag:**
- *S. dysenteriae* - usually the most severe disease
- *S. flexneri*
- *S. boydii*
- *S. sonnei*
- **Never found as normal human flora**



## APPENDIX 20-1. GENERAL CHARACTERISTICS OF SOME ENTEROBACTERIACEAE

Organism	Serologic Type(s) (Antigens)	Bacteriologic Features						Major Disease(s)	Found in Normal Flora
		Lactose	Indole	Urease	Hydrogen Sulfide	Motility	Other		
<i>Escherichia coli</i>	150+ (O, K, H)	+	+	-	-	+		Urinary tract infections; diarrhea; opportunistic	Yes
<i>Shigella dysenteriae</i>	10 (O)	-	v	-	-	-		Dysentery (type 1, severe)	No
<i>Shigella flexneri</i>	6 (O)	-	-	-	-	-		Dysentery	No
<i>Shigella boydii</i>	15 (O)	-	v	-	-	-		Dysentery	No
<i>Shigella sonnei</i>	1 (O)	-	-	-	-	-		Dysentery	No

The *Shigella* on the basis of DNA relatedness are actually variants of *E. coli*. *Shigella* possess, however, a plasmid that encodes genes which permit the bacteria to invade epithelial cells and macrophages and to replicate intracellularly. *Shigella* are **only** found in primates and **only** in the setting of enteric disease.

# Major *Shigella* Virulence Factors

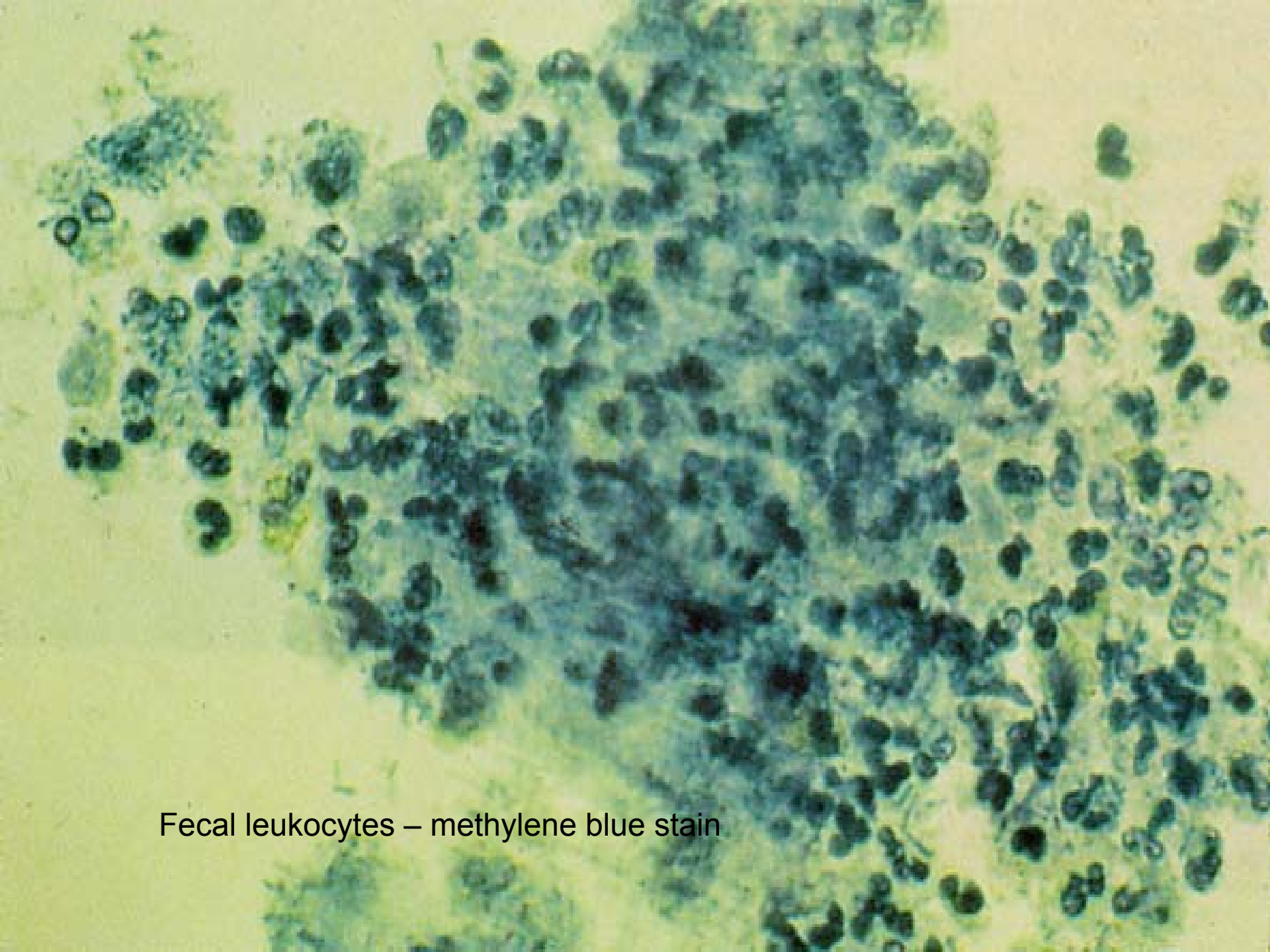
- **Invasion plasmid antigens (Ipa) - for invasion of the epithelial lining of the intestine**  
**(plasmid-encoded, secreted by a type III [contact-dependent] secretion pathway)**
  
- **Acid Resistance - survives passage through stomach to intestine**  
**(explains high infectivity / low infectious dose)**
  
- **Shiga toxin - blocks protein synthesis by inactivating 60S ribosomal subunit which causes**  
**(a) fluid secretion by blocking fluid adsorption in the intestine, and (b) more mucosal**  
**damage and bleeding.**

**Only produced by *S. dysenteriae*, which also causes most severe disease.**

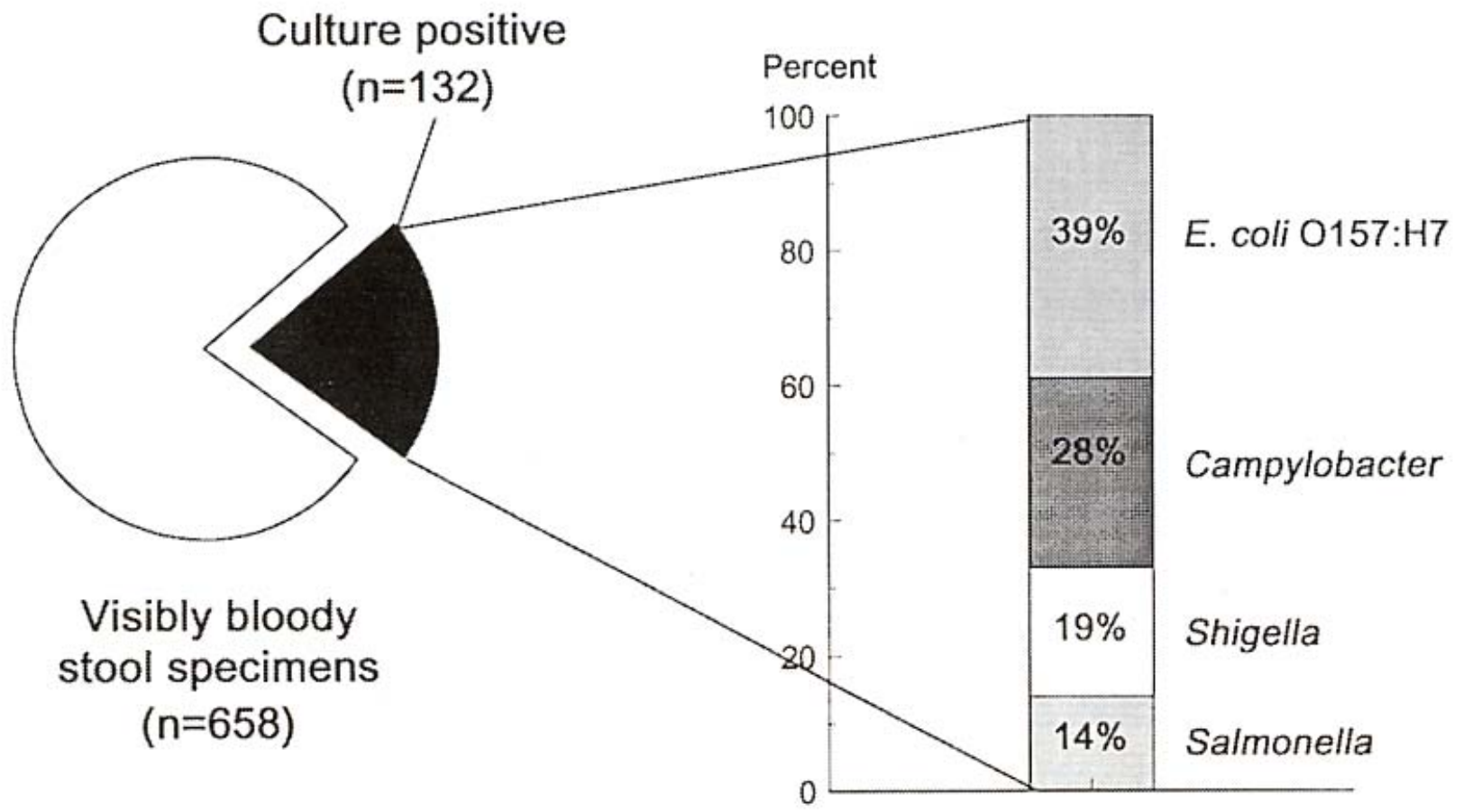
- **IcsA – cell-to-cell invasion**

The “classic”  
dysentery stool  
showing blood, mucus  
and small volume.  
Microscopically, there  
are sheets of  
leukocytes.

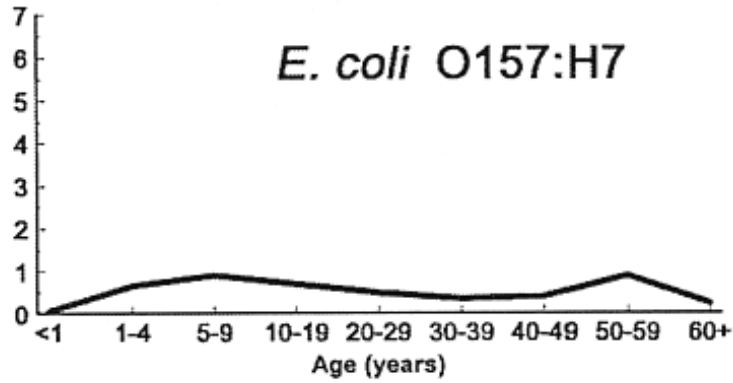




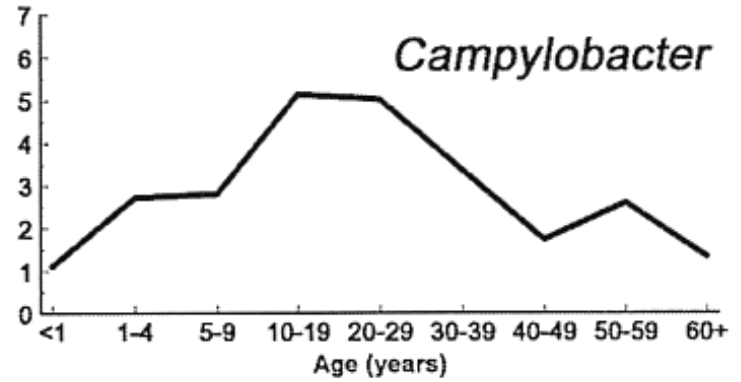
Fecal leukocytes – methylene blue stain



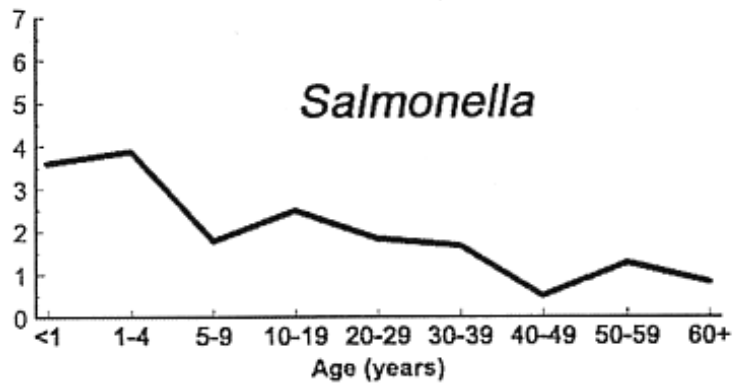
Percent of positive fecal specimens



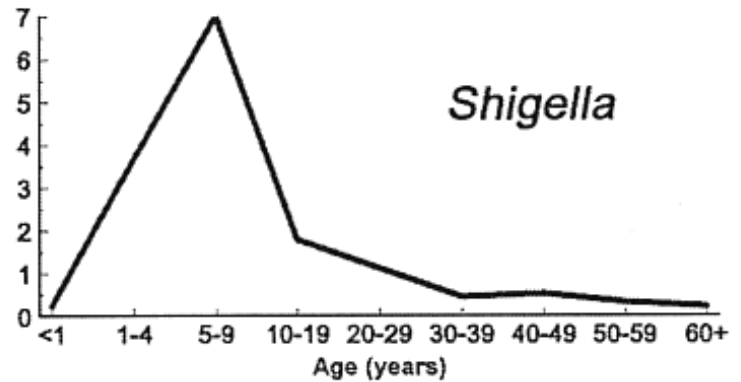
Percent of positive fecal specimens



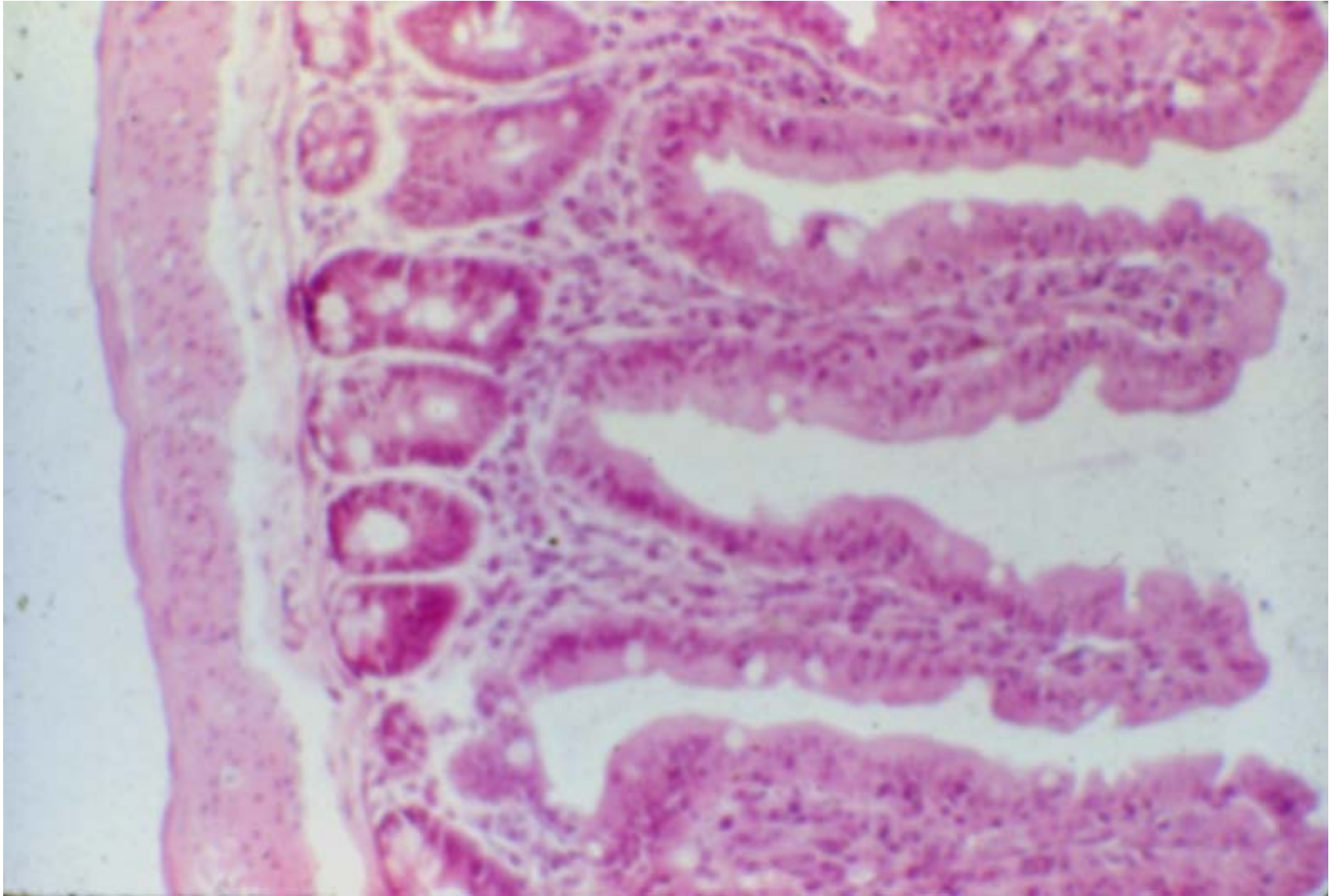
Percent of positive fecal specimens



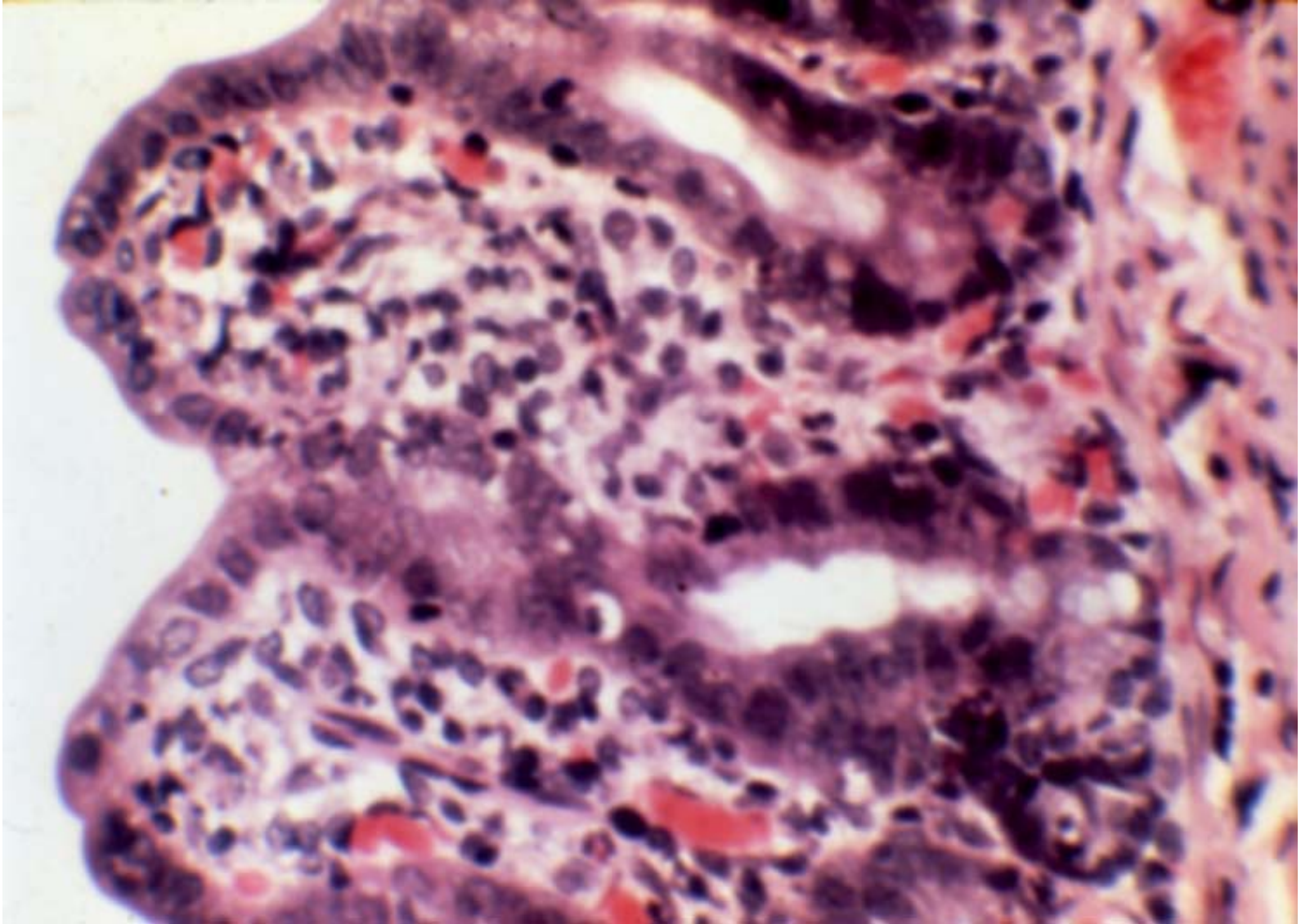
Percent of positive fecal specimens



N=30,463 fecal specimens



Normal Histology of the Colon



Histopathology of Shigellosis showing severe inflammation and blunting of the villi.



# EPIDEMIOLOGY OF SHIGELLOSIS

FOOD

FECES

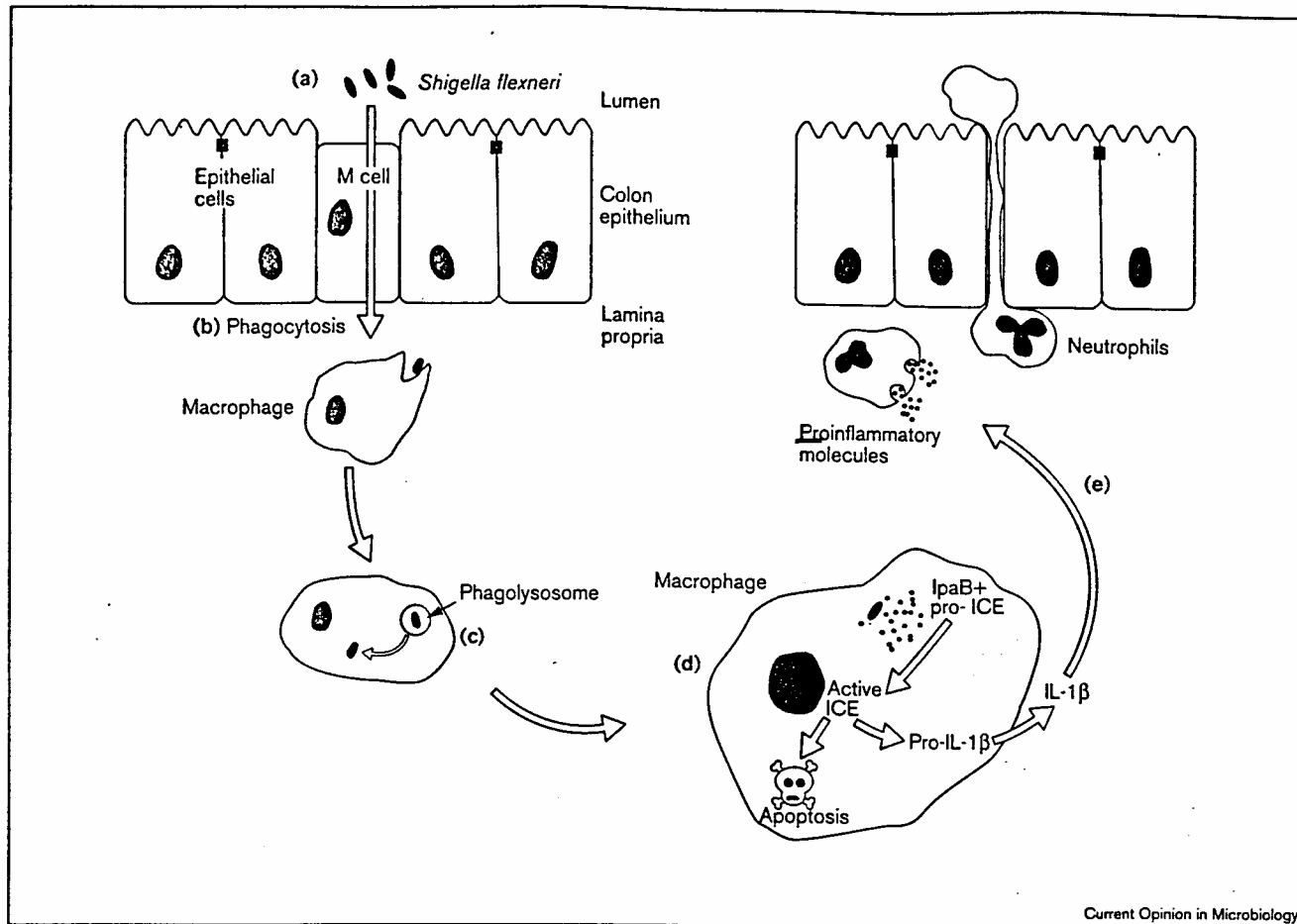
FINGERS

FLIES

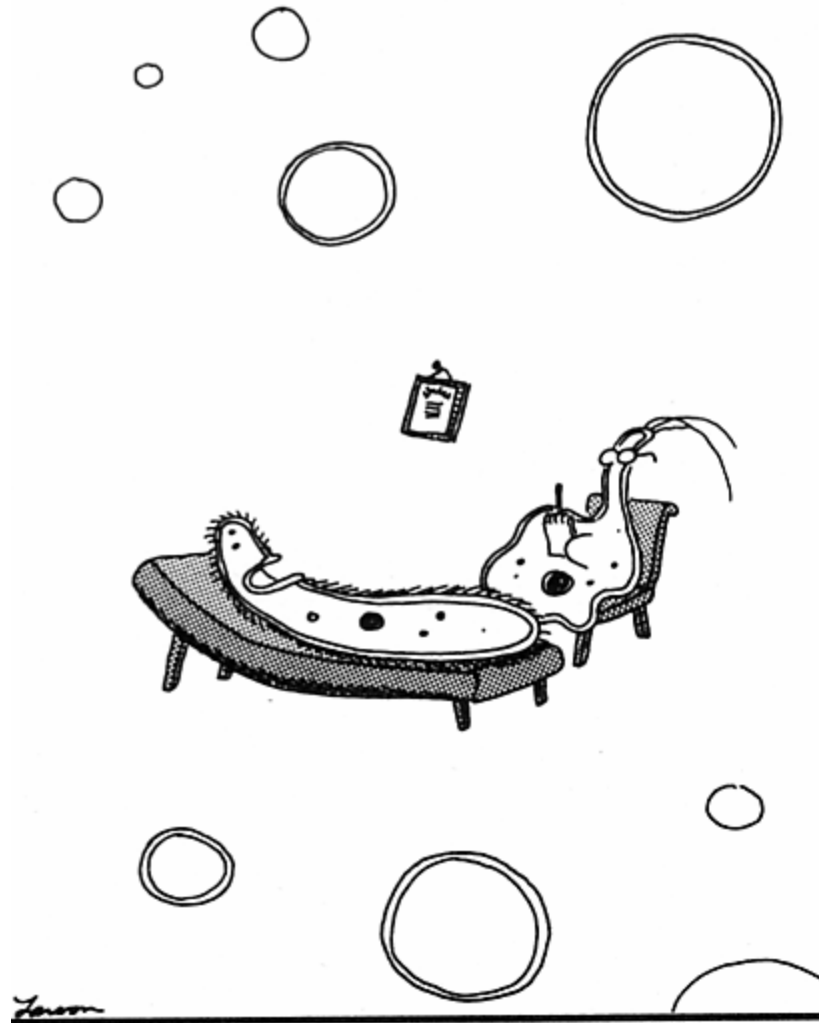
## Infectious doses of enteric pathogens

<i>Shigella</i>	$10^{1-2}$
<i>Campylobacter jejuni</i>	$10^{2-6}$
<i>Salmonella</i>	$10^5$
<i>Escherichia coli</i>	$10^8$
<i>Vibrio cholerae</i>	$10^8$
<i>Giardia lamblia</i>	$10^{1-2}$ cysts
<i>Entamoeba histolytica</i>	$10^{1-2}$ cysts

# Apoptosis and Shigella



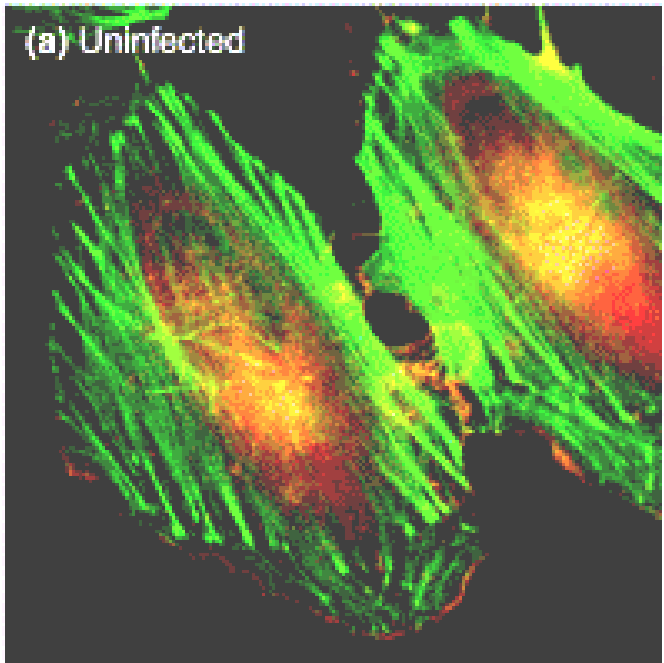
*Shigella flexneri* induced macrophage apoptosis initiates inflammation. (a) *Shigella flexneri* crosses the colonic epithelium by subverting the ability of M cells to translocate luminal antigens. (b) In the lamina propria, *S. flexneri* is phagocytosed by resident tissue macrophages in close proximity to the M cells. (c) After phagocytosis the bacteria escape from the phagolysosome and gain access to the cytoplasm of the macrophage. (d) *S. flexneri* secrete IpaB which directly interacts with ICE leading to cleavage of immature IL-1 $\beta$  to produce the biologically active, mature form of the cytokine. Concomitantly, the macrophage undergoes apoptosis and IL-1 $\beta$  is released into the lamina propria. (e) IL-1 $\beta$  acts as a potent chemotactic stimulus for the infiltration of neutrophils into the inflamed lamina propria. The neutrophils enhance inflammation by releasing proinflammatory molecules and disrupt the integrity of the epithelial barrier through transmigration into the colonic lumen.



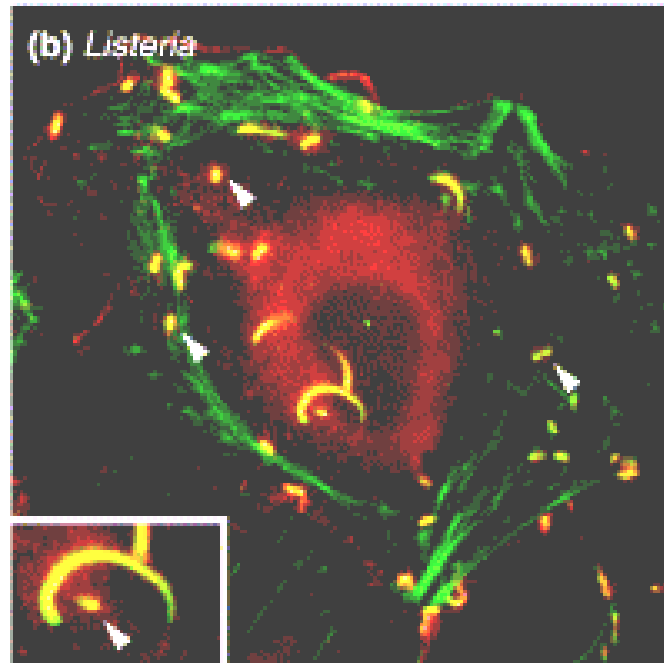
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"Well, I just feel like I'm living under  
a microscope."

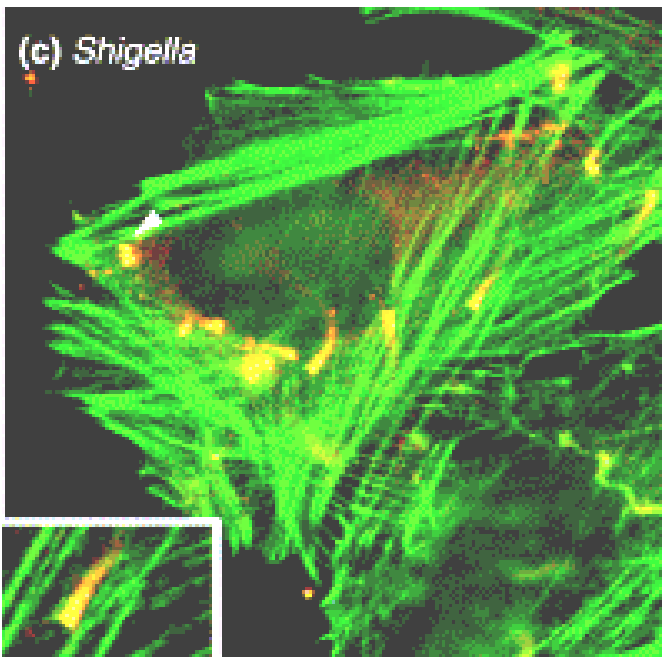
(a) Uninfected



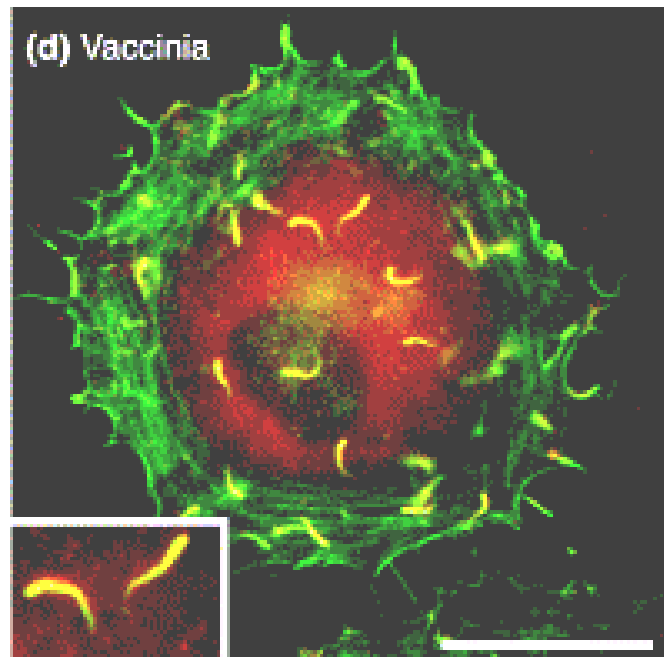
(b) *Listeria*



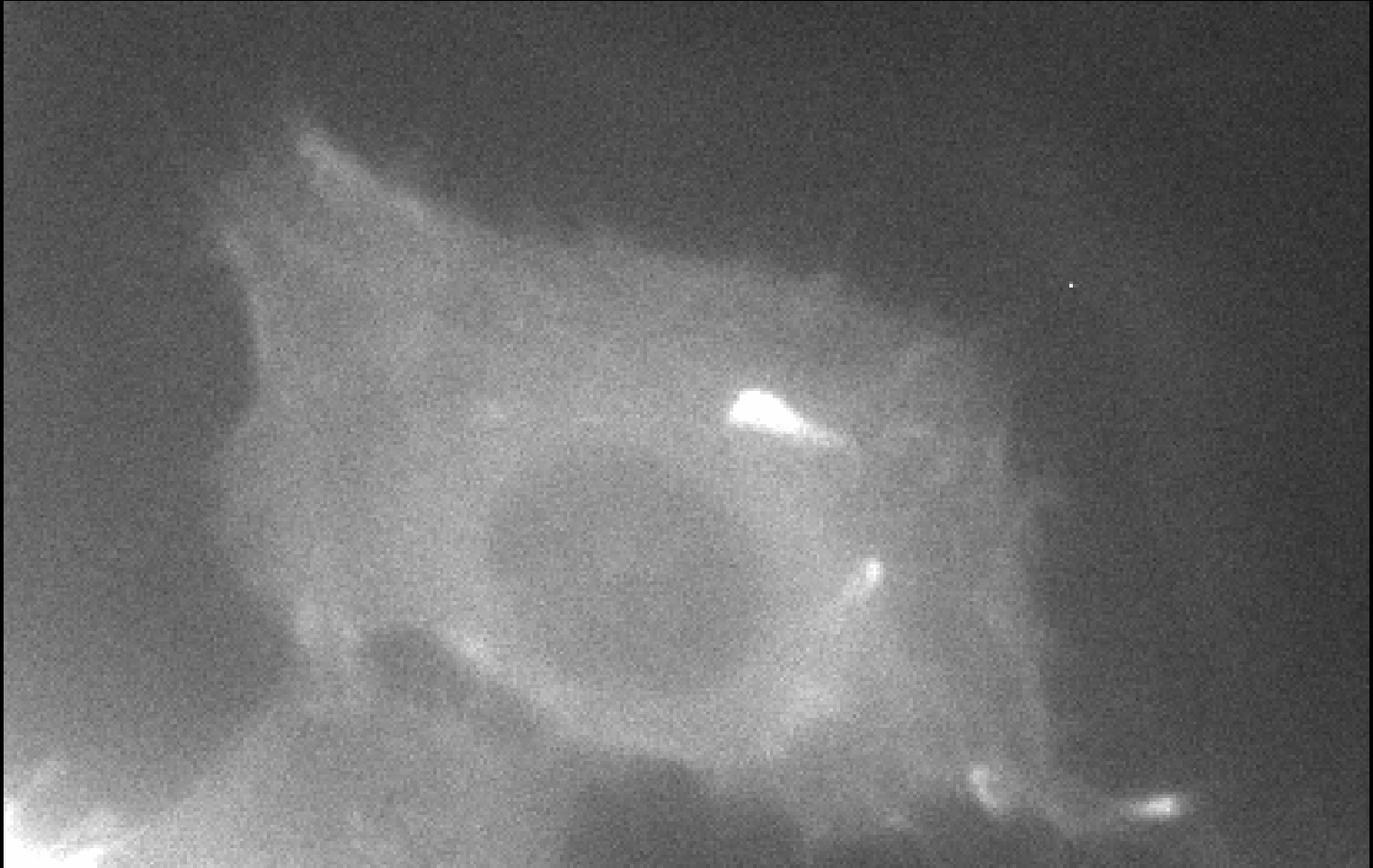
(c) *Shigella*



(d) *Vaccinia*



## *Shigella* Spreads Intercellularly by Using Actin-based Motility



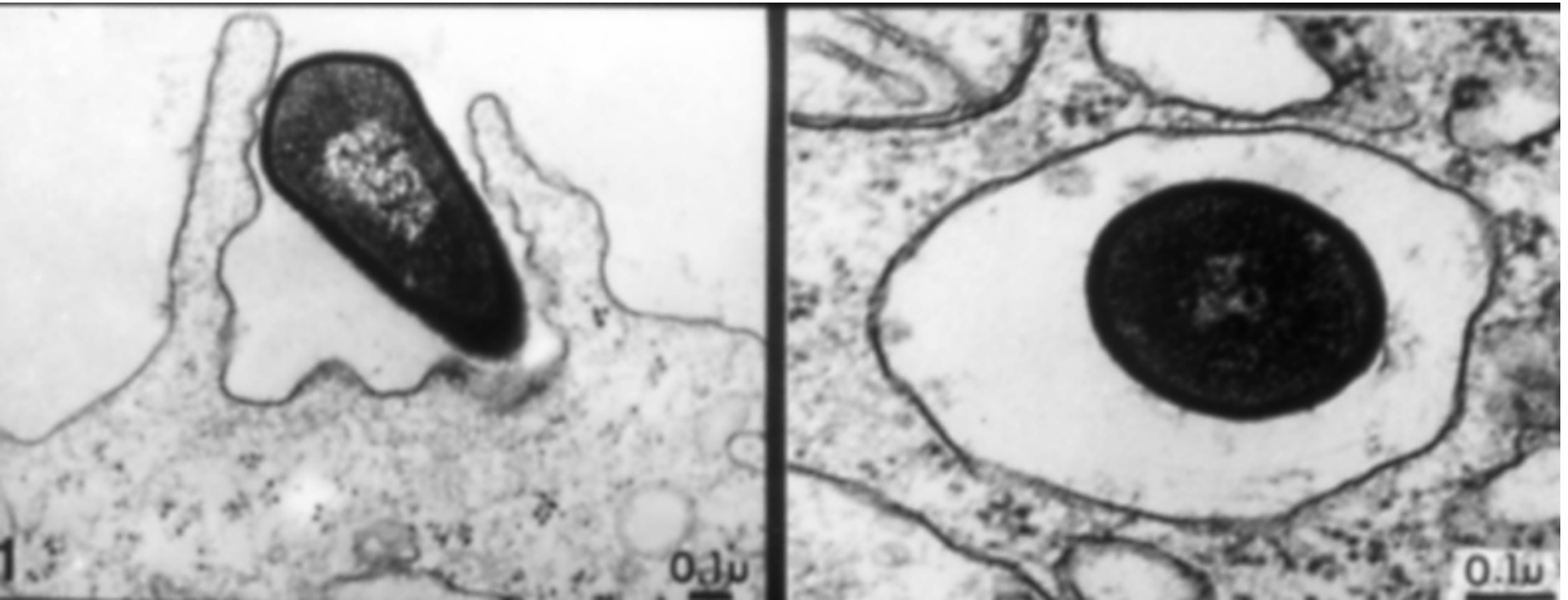
*Listeria* also spreads from cell-to-cell by actin-based motility

# *Listeria monocytogenes*

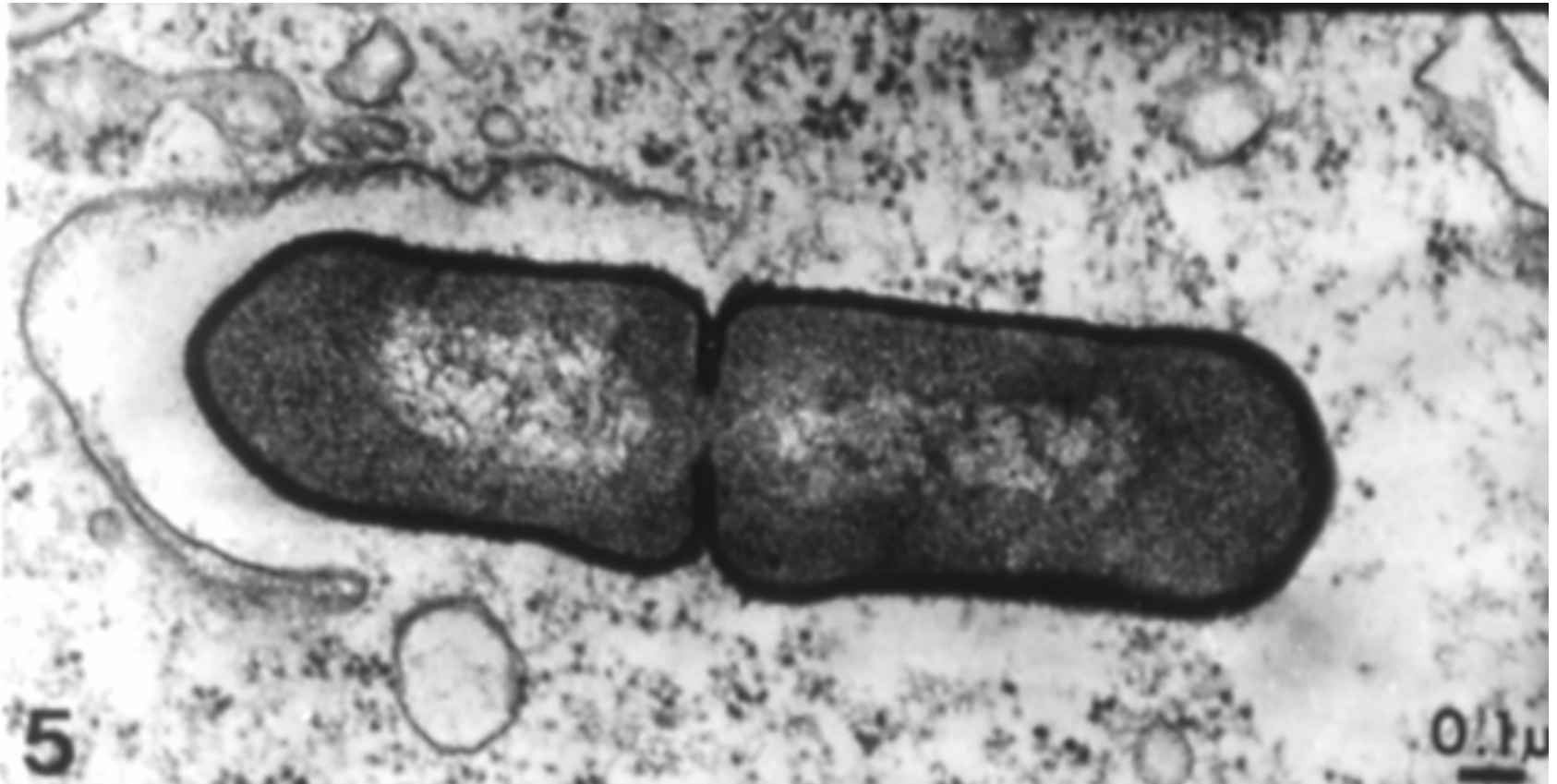
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- gram-positive, facultative, highly motile rod
- food-borne pathogen
- grows @ 4° C (food processors nightmare)
- avg. incubation period is 30 days
- most deaths involve fetuses, newborns, or immunocompromised
- one of few bacteria that can cross the placenta
- 5% TO 10% of human pop. carry in intestinal tract asymptotically





Listeria enters epithelial cells by attachment to a specific receptor Mediated by the bacterial protein internalin. The bacterium is Initially in an intracellular compartment surrounded by a host membrane



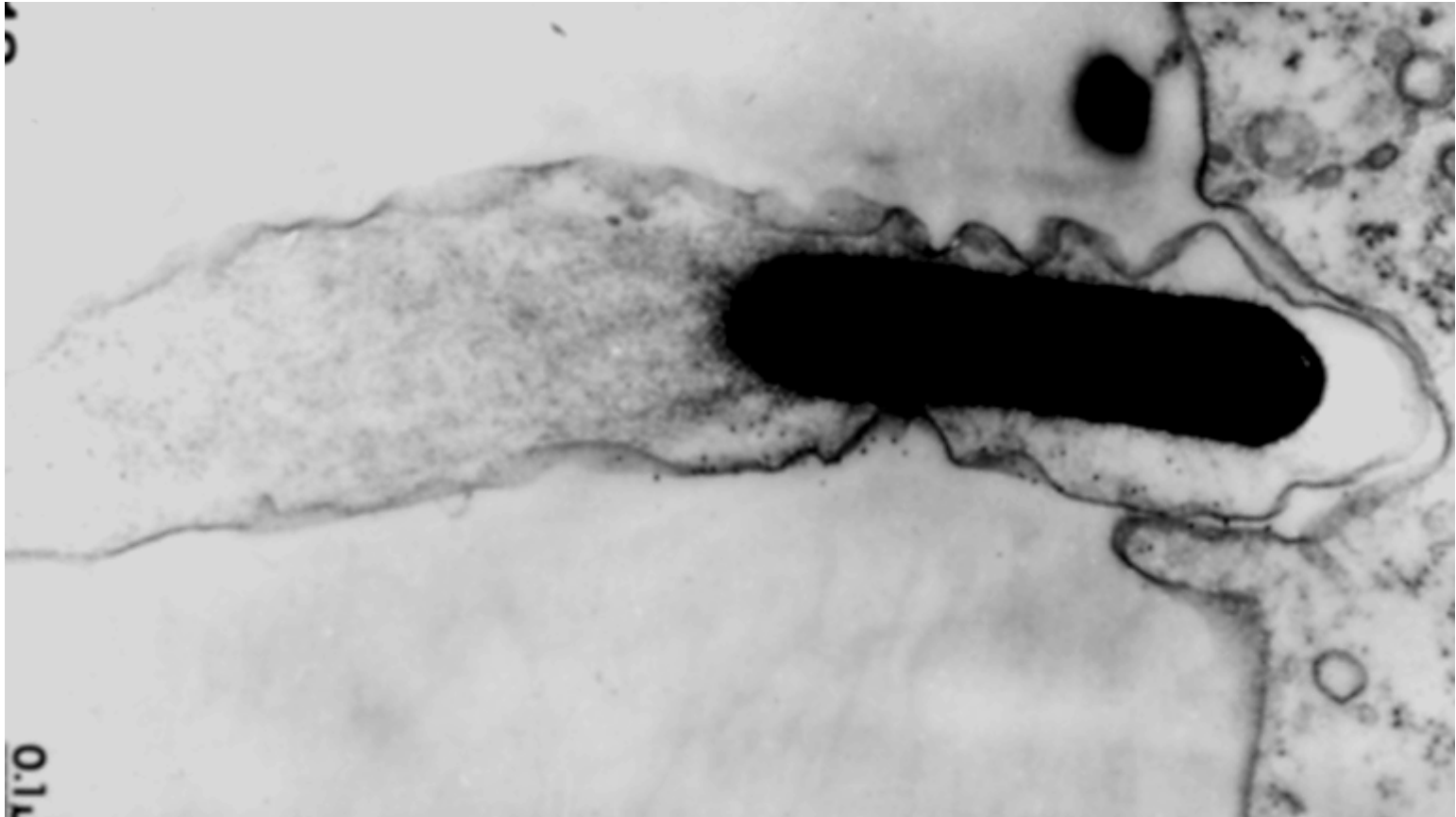
Within minutes after entry the bacterium secretes an enzyme which destroys the surrounding host membrane releasing the Microbe into the host cell cytoplasm.



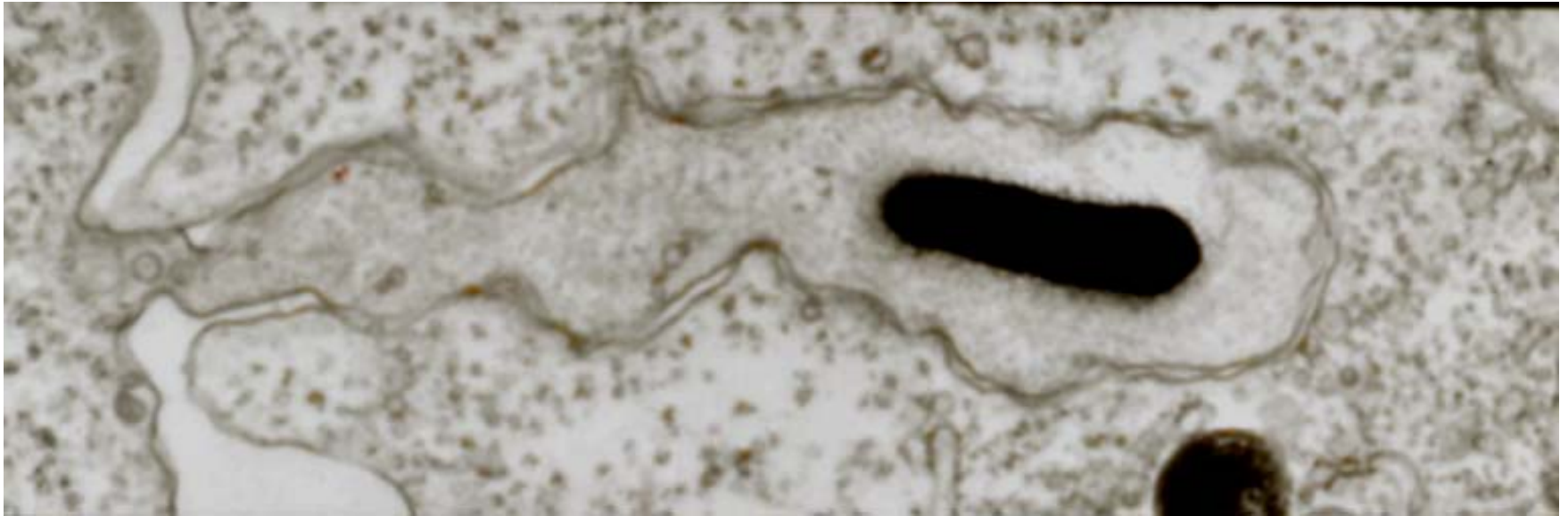
Once in the host cell cytoplasm, the bacterium secretes a protein that localizes to one pole of the organism and acts as a nucleation point for host cell actin polymerization. The microbes move through the cytoplasm driven by the polymerization energy.



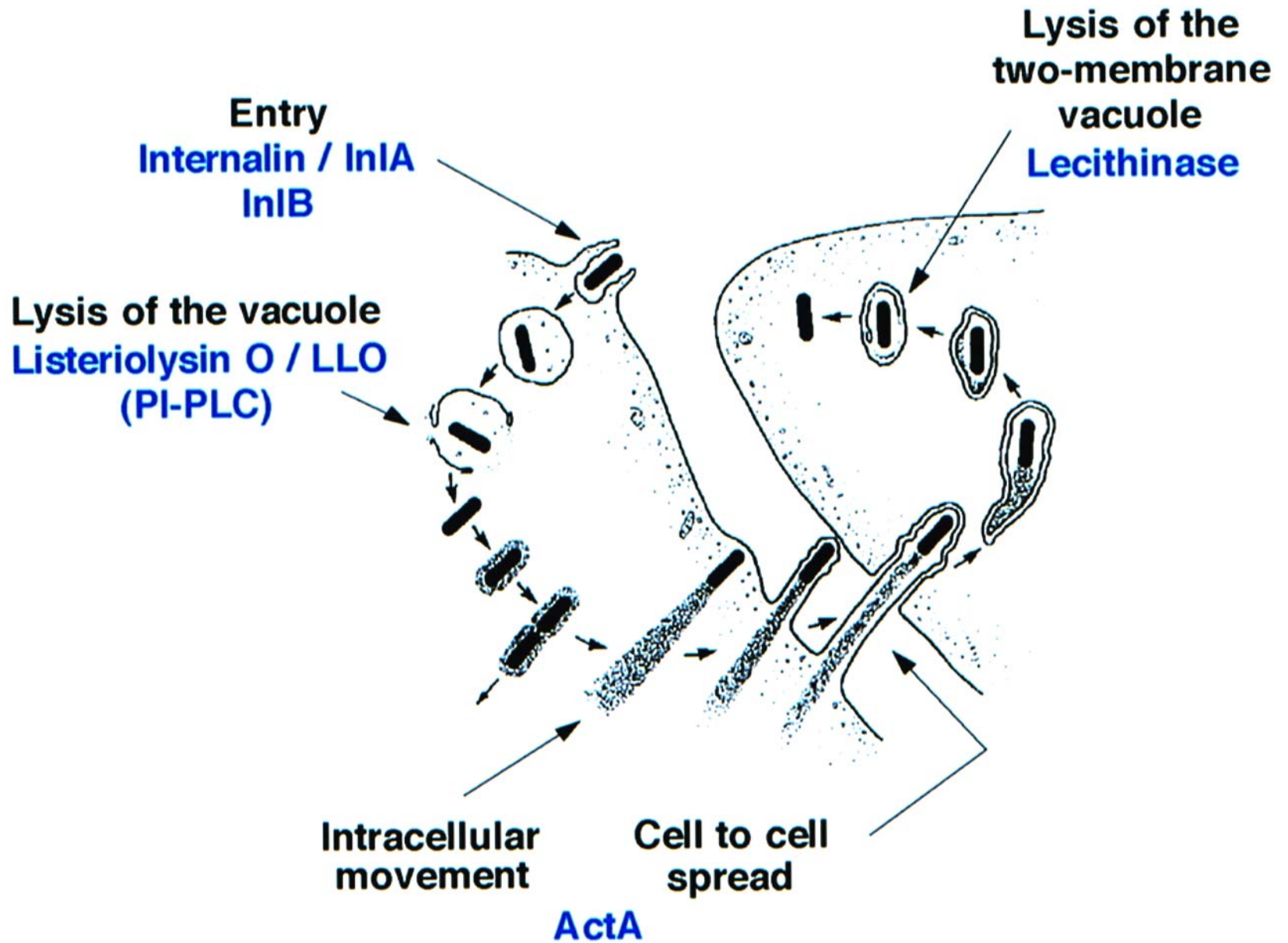
As the bacteria move to the host cell surface, some are thrust out as projections. One can see the polymerized “tail” of actin.



If the projections come into contact with an adjacent uninfected cell, the projection may enter by a phagocytic event. In this way, the microbe and its progeny can move from host cell-to-cell.



Listeria projections are taken up by adjacent cells and the entering bacteria can be seen surrounded by a double membrane. This membrane is destroyed by a bacterial-mediated enzyme which releases the bacterium into the cytoplasm so the cycle can begin again. Thus, the organism spreads from cell-to cell without becoming extracellular.



# Three Types of Enteric Infection

	I	II	III
Mechanism	Noninflammatory (enterotoxin)	Inflammatory (invasion ?cytotoxin)	Penetrating
Location	proximal small bowel	colon	distal small bowel
Illness	Watery diarrhea	Dysentery	Enteric fever
Stool exam	No fecal leukocytes	Fecal polymorphonuclear leukocytes	fecal mononuc. Lymph.
Examples	<i>Vibrio cholerae</i> <i>Escherichia coli</i> (LT) <i>E. coli</i> (ST) <i>Clostridium perfringes</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i> Rotavirus <i>Giardia lamblia</i> Norwalk-like viruses Cryptosporidium	<i>Shigella</i> Invasive <i>E. coli</i> <i>Salmolnella enteritidis</i> <i>V. parahemolyticus</i> <i>Clostridium difficile</i> <i>Campylobacter jejuni</i> <i>Entamoeba histolytica</i>	<i>Salmonella typhi</i> <i>Yersinia enterocolitica</i> <i>Campylobacter fetus</i>



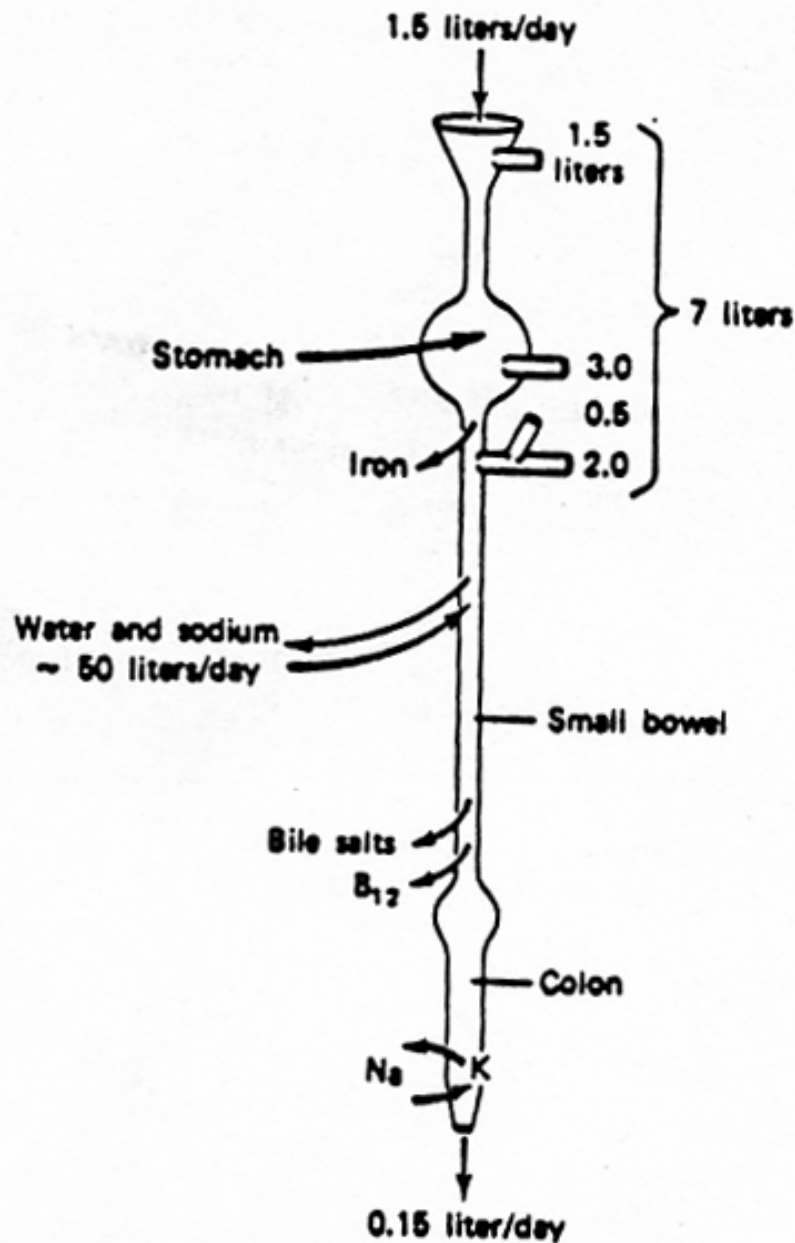
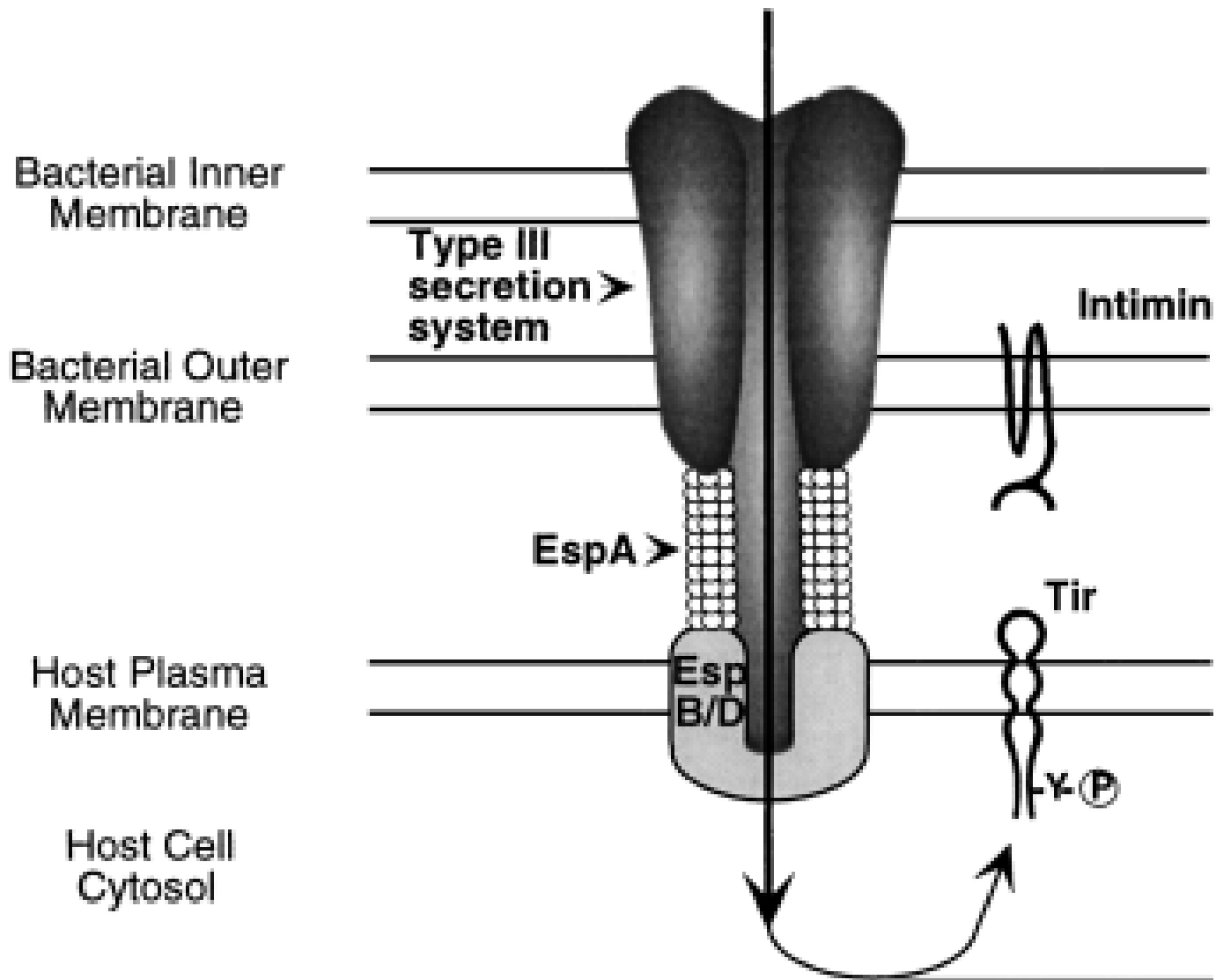


Diagram of fluid balance in the healthy adult gastrointestinal tract.

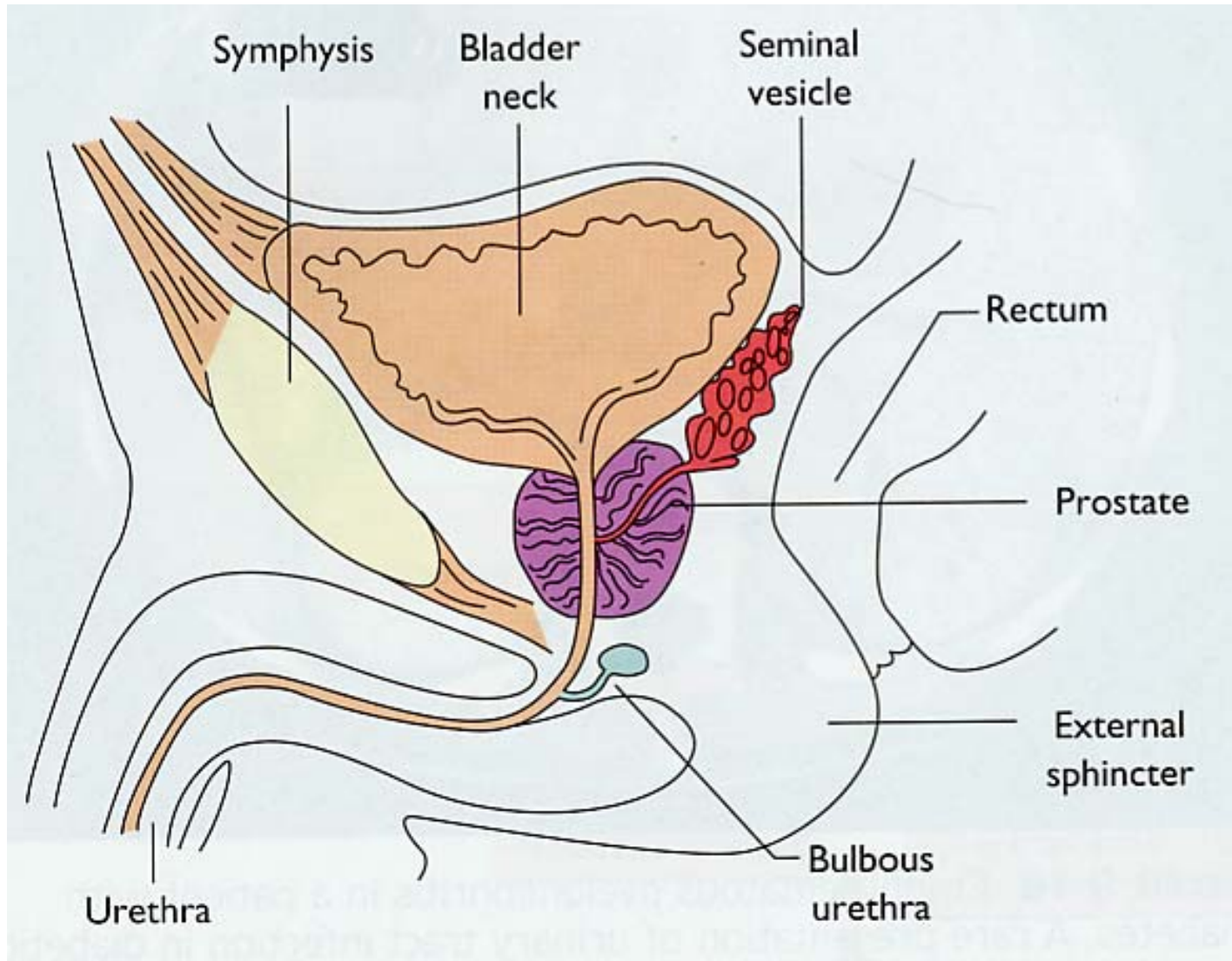
**TABLE 5.** Three Types of Enteric Infection

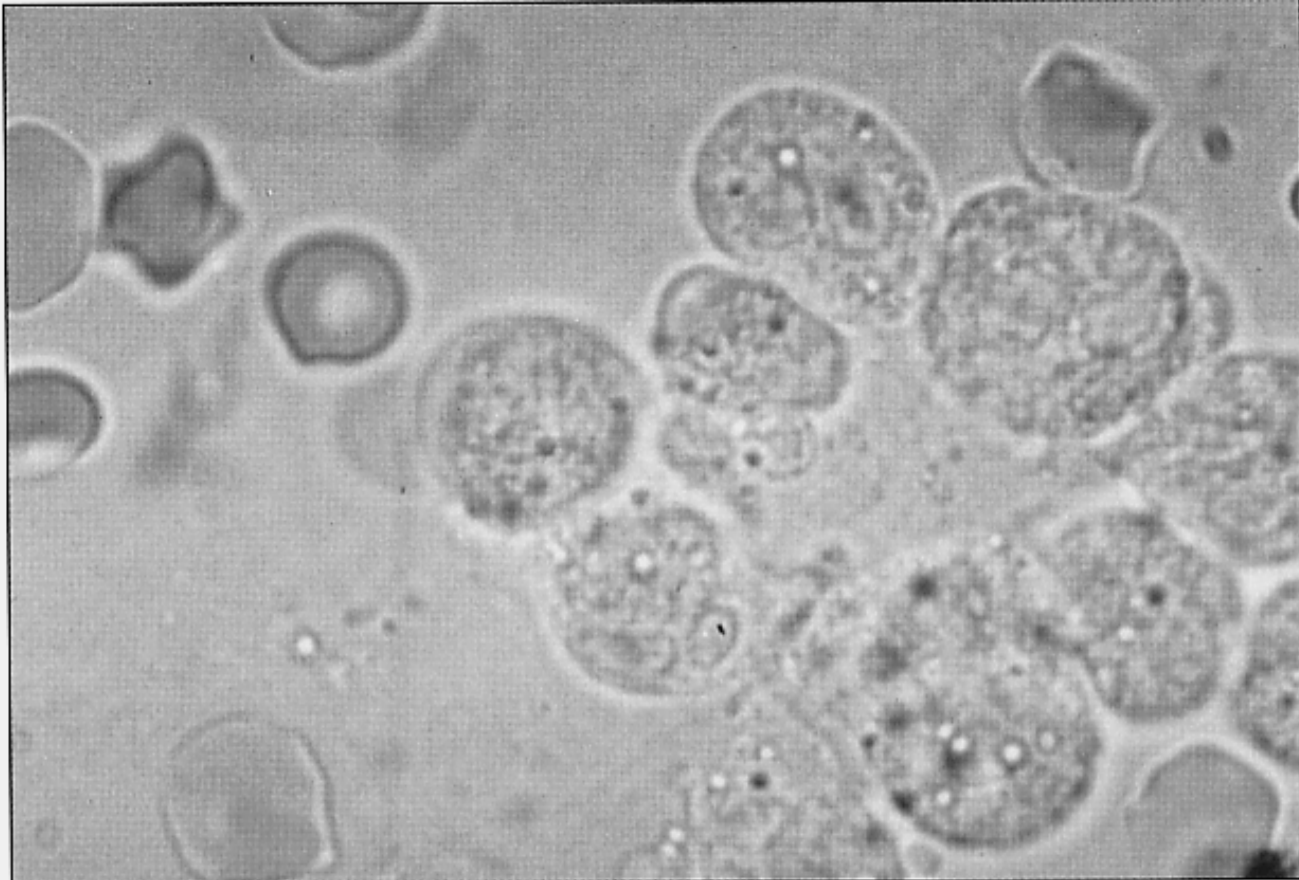
	I	II	III
Mechanism	Noninflammatory (enterotoxin)	Inflammatory (invasion ?cytotoxin)	Penetrating
Location	Proximal small bowel	Colon	Distal small bowel
Illness	Watery diarrhea	Dysentery	Enteric fever
Stool examination	No fecal leukocytes	Fecal polymorphonuclear leukocytes	Fecal mononuclear leukocytes
Examples	<i>Vibrio cholerae</i> <i>Escherichia coli</i> (LT) <i>E. coli</i> (ST) <i>Clostridium perfringens</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i> ? <i>Salmonella</i> ? <i>Vibrio parahemolyticus</i> <i>Giardia lamblia</i> Rotavirus Norwalk-like viruses <i>Cryptosporidium</i>	<i>Shigella</i> Invasive <i>E. coli</i> <i>Salmonella enteritidis</i> <i>V. parahemolyticus</i> ? <i>Clostridium difficile</i> ? <i>Campylobacter jejuni</i> <i>Entamoeba histolytica</i> <sup>a</sup>	<i>Salmonella typhi</i> <i>Yersinia enterocolitica</i> ? <i>Campylobacter fetus</i>

<sup>a</sup> Although amebic dysentery involves tissue inflammation, the leukocytes are characteristically pyknotic or absent, having been destroyed by the virulent amebae.<sup>345</sup>

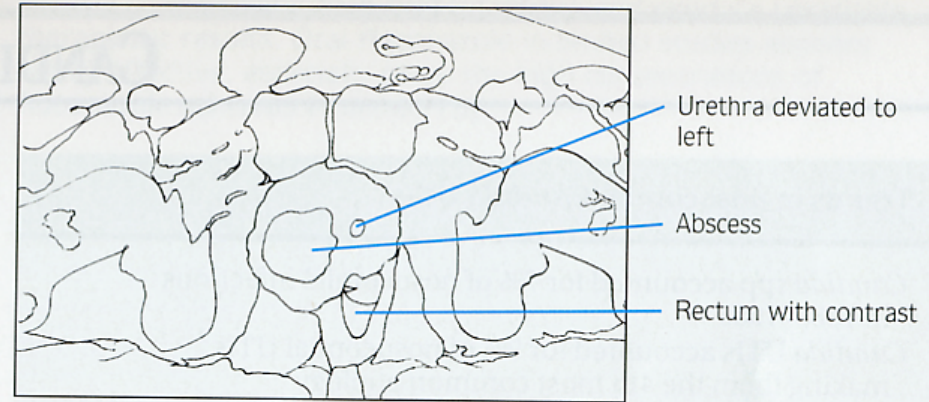
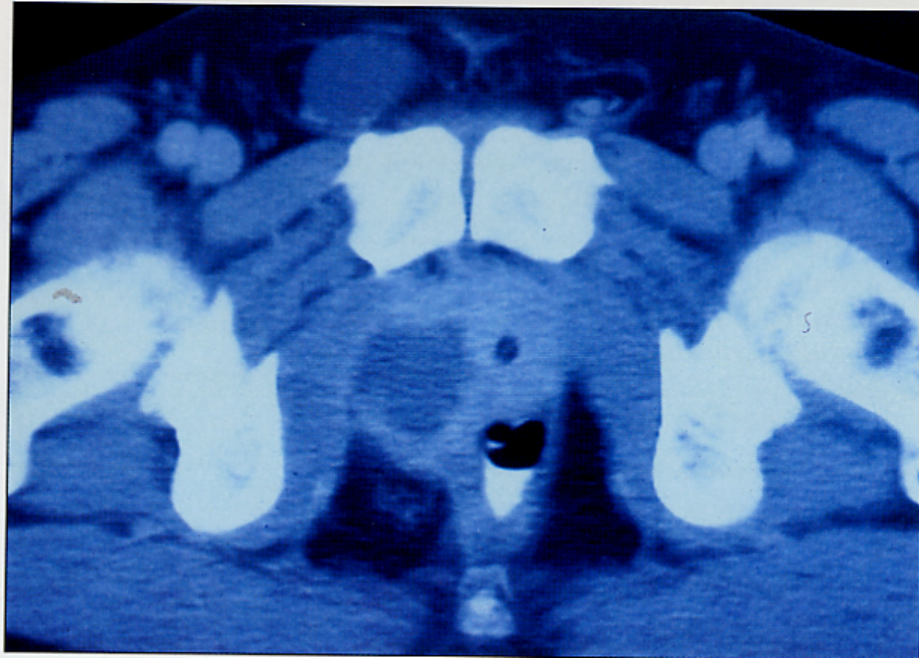


The type III secretion system of EPEC used to deliver virulence factors, including Tir, into the host cell cytosol or membrane. Several gram-negative pathogens use this conserved secretion system to deliver diverse effectors into host cells to mediate several different effects within mammalian and even plant cells.





Pyuria in acute bacteria prostatitis. Pyuria is the presence of leukocytes in the voided urine. This inflammatory response is due to concurrent cystitis that accompanies the prostatitis. Pyuria is not specific, of course, but is always a feature of untreated acute bacterial prostatitis.



CAT scan demonstrating a prostatic abscess. Patients with diabetes, indwelling catheters, immunocompromised status, urinary tract instrumentation and on maintenance hemodialysis are especially prone to the development of prostatic abscess. Clinical symptoms include Acute urinary retention, fever, Dysuria, urinary frequency and perineal pain. ***E. coli* is the predominant pathogen isolated from such lesions.**

## Cardinal clinical manifestations of chronic bacterial prostatitis

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Most common cause of relapsing urinary tract infection in men

Asymptomatic periods between episodes of recurrent bacteriuria

Obstruction or irritative voiding symptoms (occasional)

Vague discomfort in pelvis and perineum (infrequent)

Physical findings on palpation normal

Expressed prostatic secretions or postmassage urine culture needed for precise diagnosis

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*Salmonella typhi*

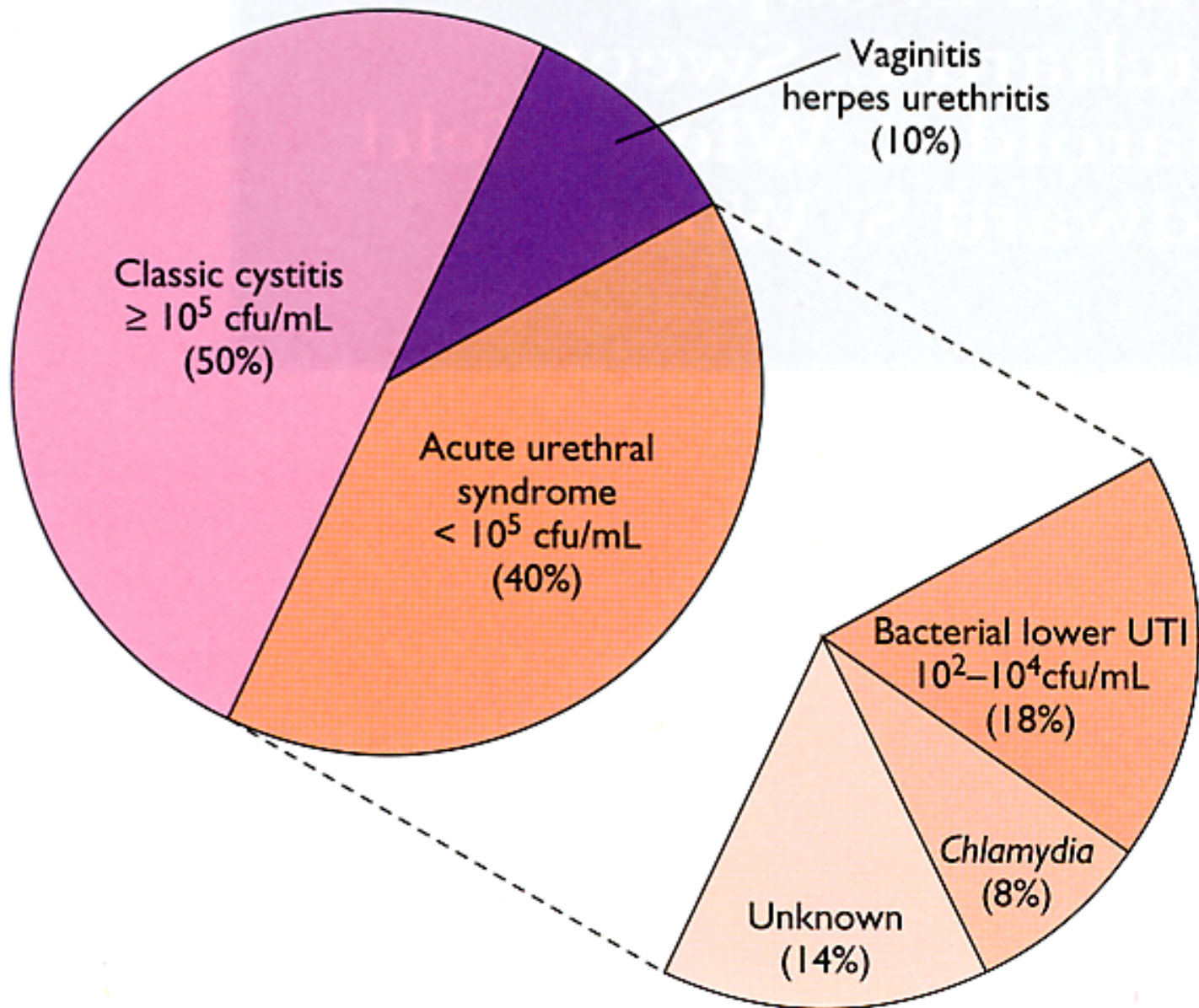
## APPENDIX 20-2. EXOTOXINS PRODUCED BY ENTEROBACTERIACEAE

Toxin	Target	Enzymatic Activity	Primary Action	Effect	Genetic Control
<i>Escherichia coli</i> LT	G regulatory protein	ADP-ribosylation	Adenylate cyclase stimulation	Fluid loss	Plasmid
<i>Escherichia coli</i> ST	Glycoprotein receptor	Unknown	Guanylate cyclase stimulation	Fluid loss	Plasmid
<i>Shigella dysenteriae</i> , type 1 Shiga toxin	60S ribosome	Modifies 28S ribosomal RNA	Inhibits protein synthesis	Cell death	Chromosomal
Shigalike toxins	60S ribosome	Modifies 28S ribosomal RNA	Inhibits protein synthesis	Cell death	Temperate phage

## Distinguishing Dysuria

TABLE 112-3 ■ Factors That Distinguish Acute Cystitis From Vaginitis and Urethritis

FACTOR	ACUTE CYSTITIS	ACUTE URETHRITIS	VULVOVAGINITIS
<i>Pathogen</i>	<i>E. coli</i> , <i>S. saprophyticus</i>	<i>C. trachomatis</i> , <i>N. gonorrhoeae</i> , herpes simplex virus	<i>Candida</i> , <i>Trichomonas</i> , nonspecific vaginosis
<i>Symptoms</i>	Internal dysuria, frequency, urgency, hematuria	Internal dysuria, frequency, urgency, vaginal discharge	External dysuria, vaginal discharge, vaginal odor
<i>Onset</i>	Abrupt; symptoms severe	Gradual; symptoms mild	Gradual; symptoms mild
<i>History</i>	Prior UTI, diaphragm use	New sexual partner	Dyspareunia
<i>Physical findings</i>	Suprapubic tenderness	Cervicitis	Vulvovaginitis



## Estimated Daily Discharge of *E. coli* in Feces

Item	Human	Cow	Hog	Sheep	Chicken
Moisture Content %	77	83	67	74	72
Avg Wgt of 24 hr fecal discharge wet weight in gr.	150	26,500	2700	1130	182
Coliforms/ gr.x $10^{-9}$	13	.23	3.3	16	1.1
Total coliforms discharged per day x $10^{-9}$	1.95	5.43	8.91	18	.24



Severe Dehydration 10-15% of body weight loss  
Peripheral circulatory failure, peripheral pulse absent, apathy, cold extremities