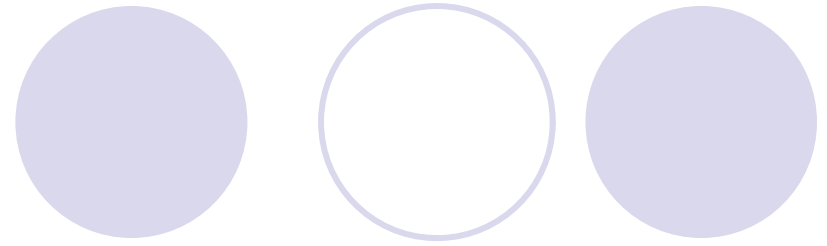


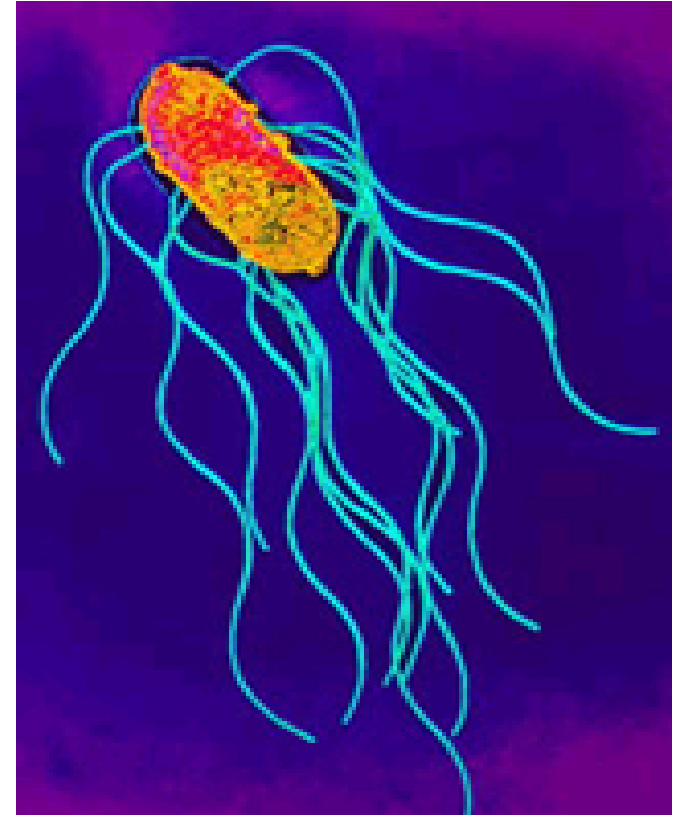
Enterobacteriaceae - Salmonella

Dr. Jayshri D Pethani
Professor of Microbiology

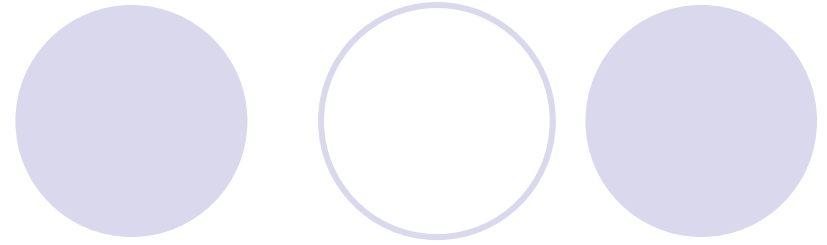
Morphology



- Gram negative bacilli, size 1-4 μ x 0.6 μ
- Motile with peritrichous flagella
- Non capsulated
- Non sporing
- May possess fimbriae
- Aerobic & facultative anaerobes

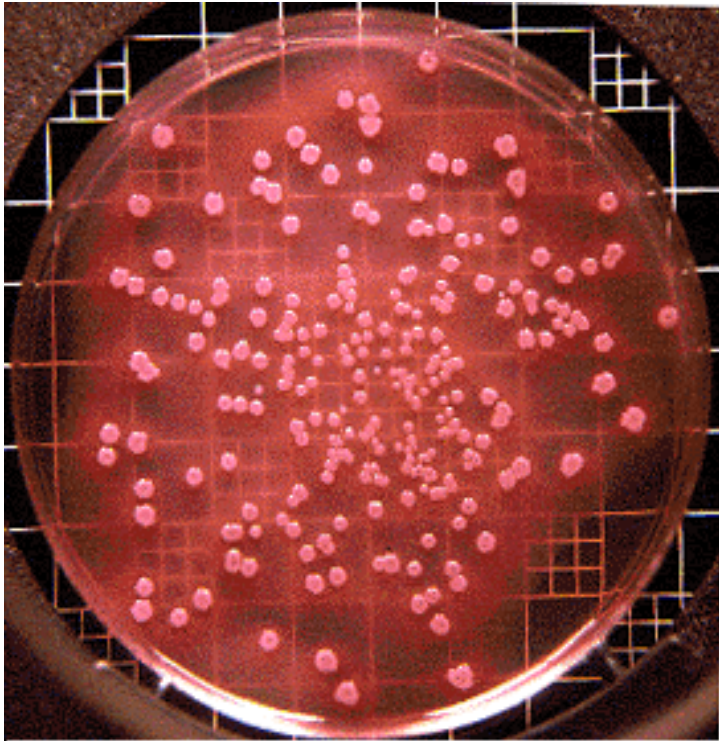


Culture characters:

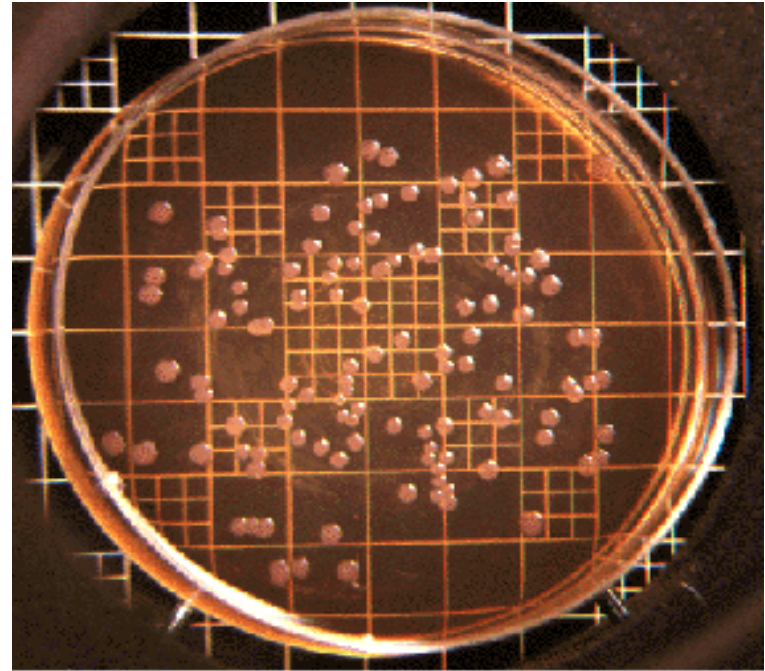


- N. agar: Large, circular, low convex, translucent & smooth
- Mac Conkey agar & DCA: Colourless & pale
- Wilson & Blair: Jet black with metallic sheen
- Selenite F & tetrathionate broth
- Salmonella – Shigella agar

Growth on MacConkey media:



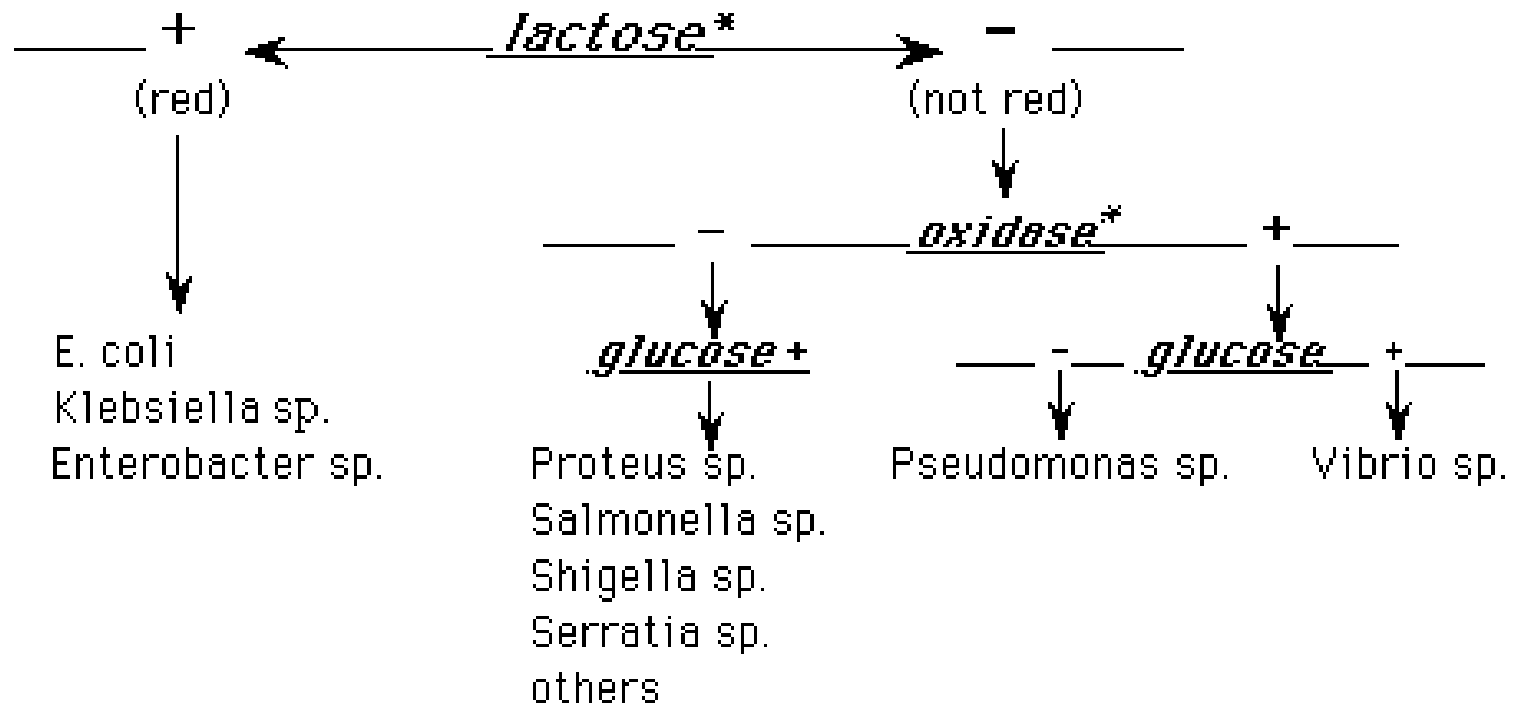
Escherichia coli
MacConkey agar



Salmonella typhi
MacConkey agar

Identification of Gram Negative Rods Growth on MacConkey agar

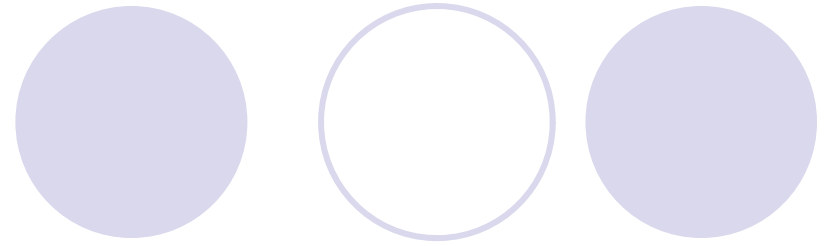
Enterics sp.*



confirm with

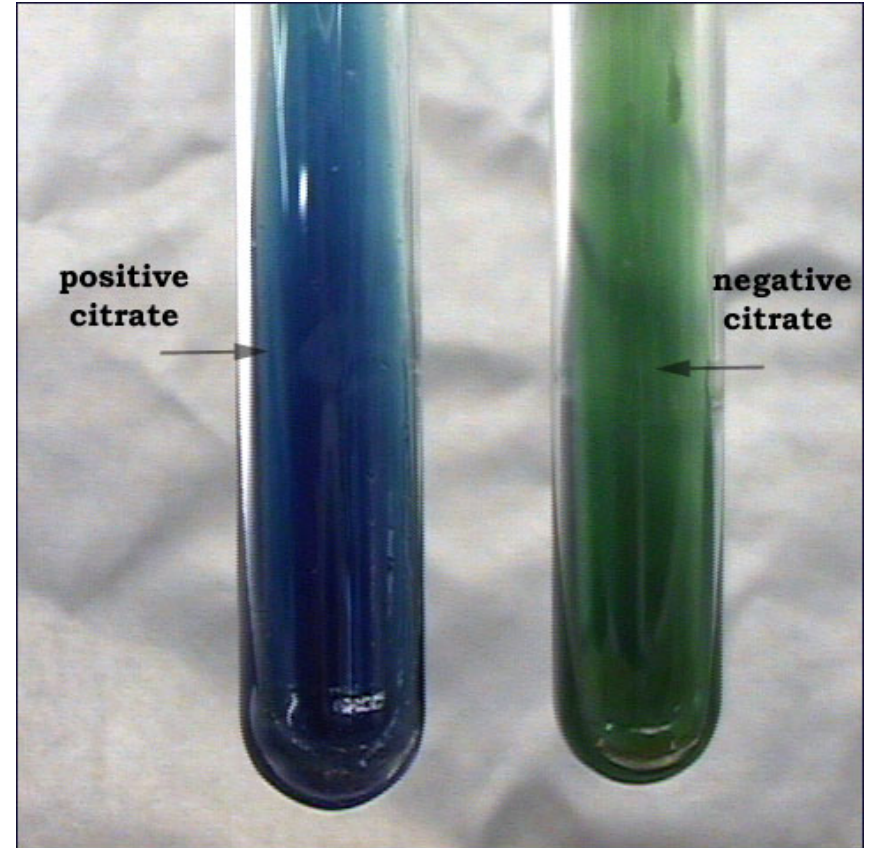
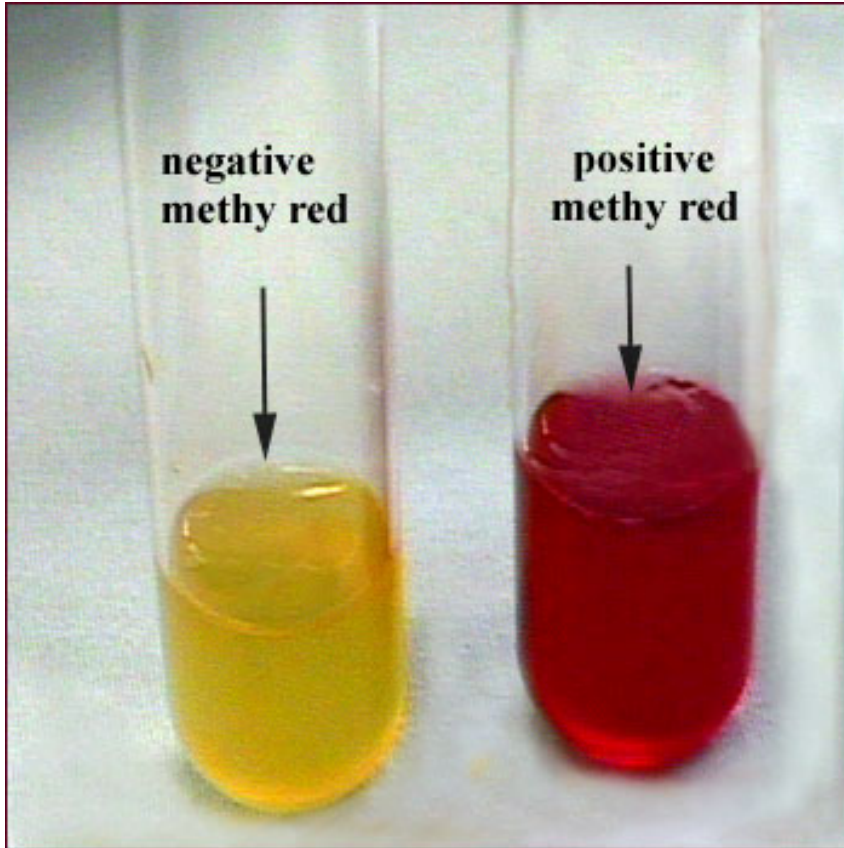
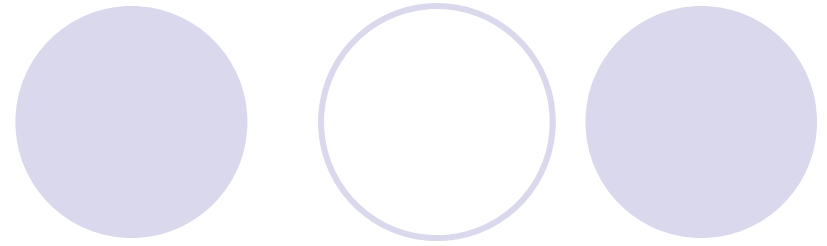
DSIA Enterotube* capsule**

Biochemical tests:

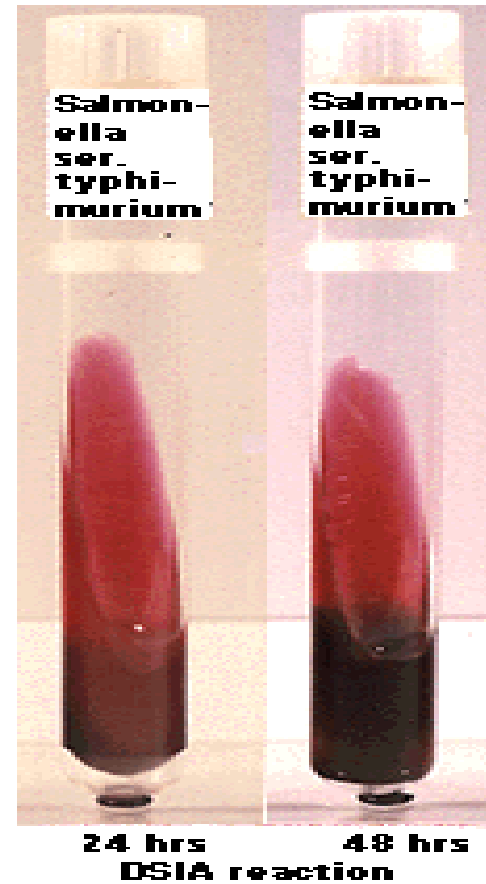
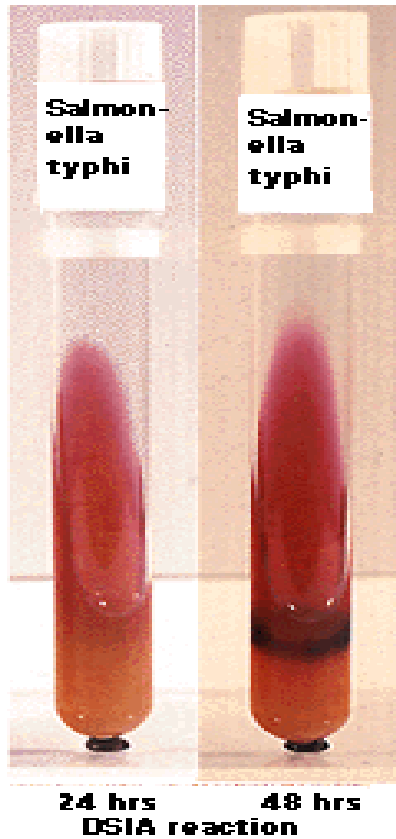


- Fermentation of sugars: Glucose, Mannitol & Maltose
- IMViC : - + - +
- Urease test: Negative
- PPA: Negative
- TSI
- Oxidase test: Negative
- Catalase test: Positive

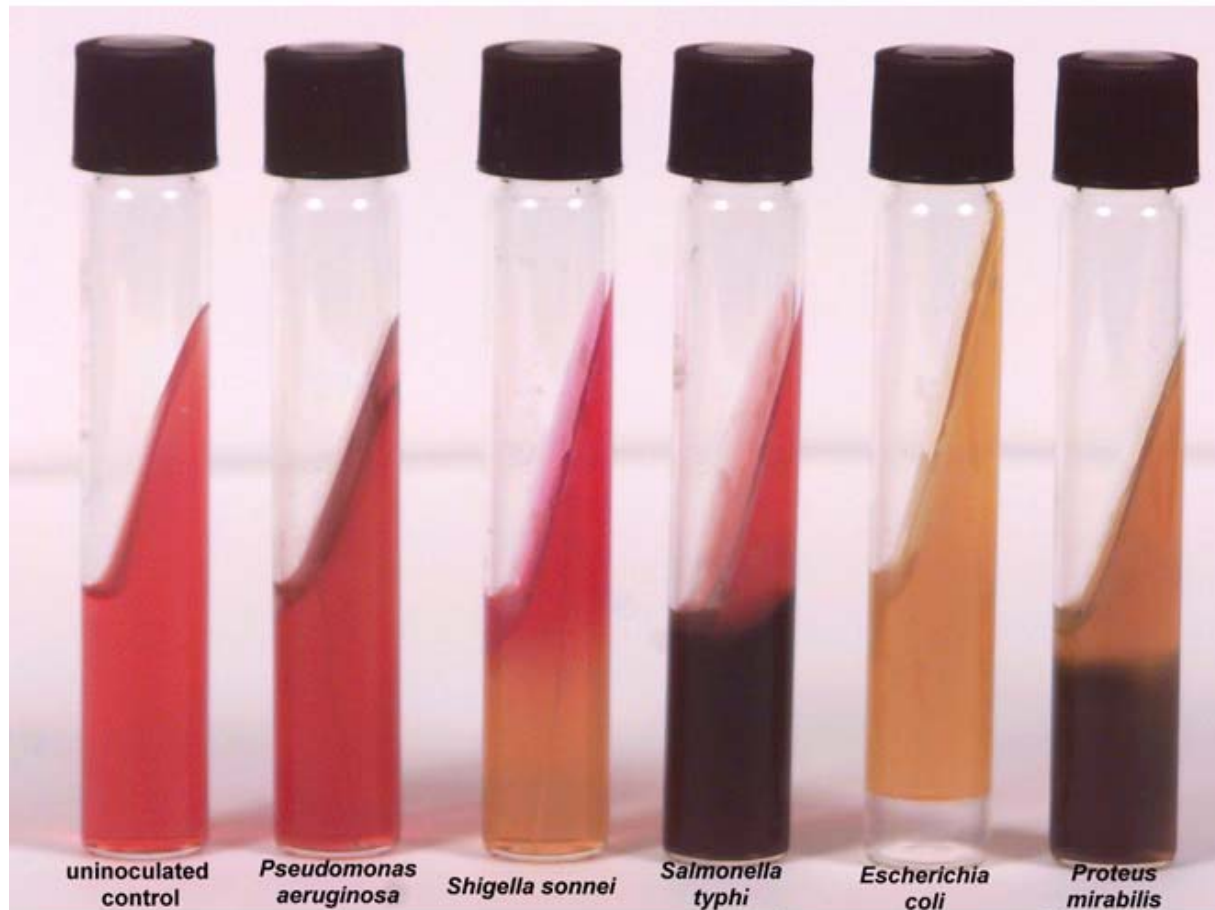
Biochemical test



TSI medium:

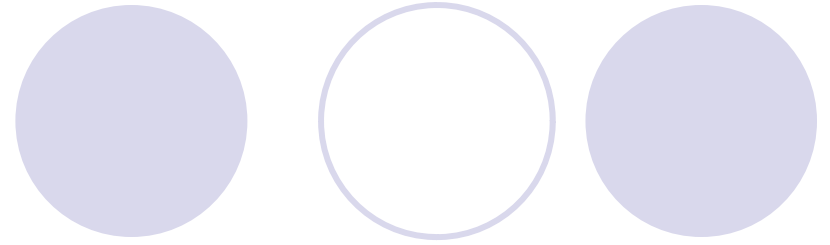


Reaction on TSI



ASM MicrobeLibrary.org©Chamberlain

Antigenic structure:



- Flagellar antigen H
- Somatic antigen O
- Surface antigen Vi

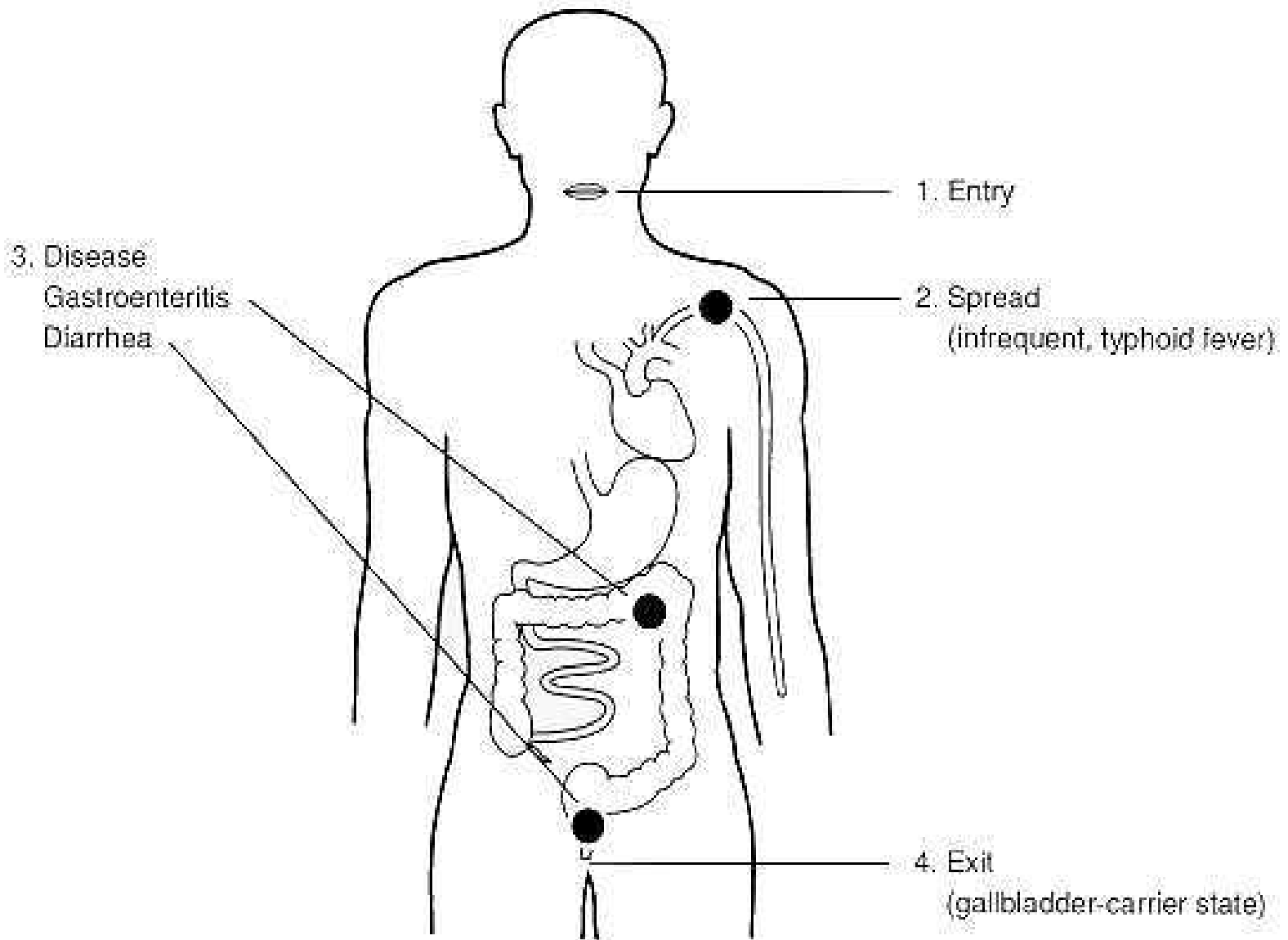
- Kaufmann – White Scheme for classification

Clinical Syndromes of Salmonella

Salmonellosis = Generic term for disease

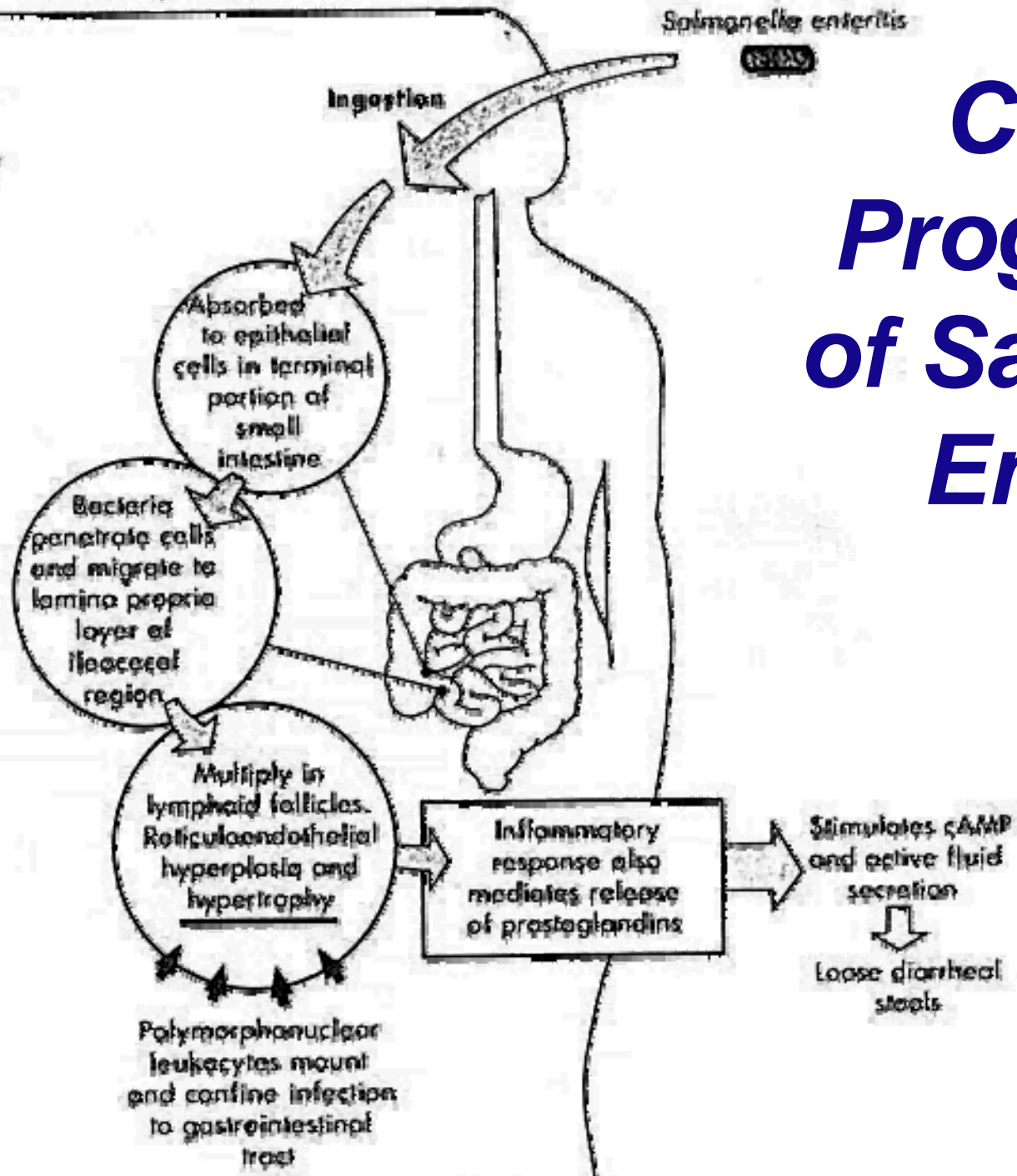
Clinical Syndromes

- **Enteritis** (acute gastroenteritis)
- **Enteric fever** (prototype is **typhoid fever** and less severe paratyphoid fever)
- **Septicemia** (particularly *S. choleraesuis*, *S. typhi*, and *S. paratyphi*)
- **Asymptomatic carriage** (gall bladder is the reservoir for ***Salmonella typhi***)





Clinical Progression of Salmonella Enteritis

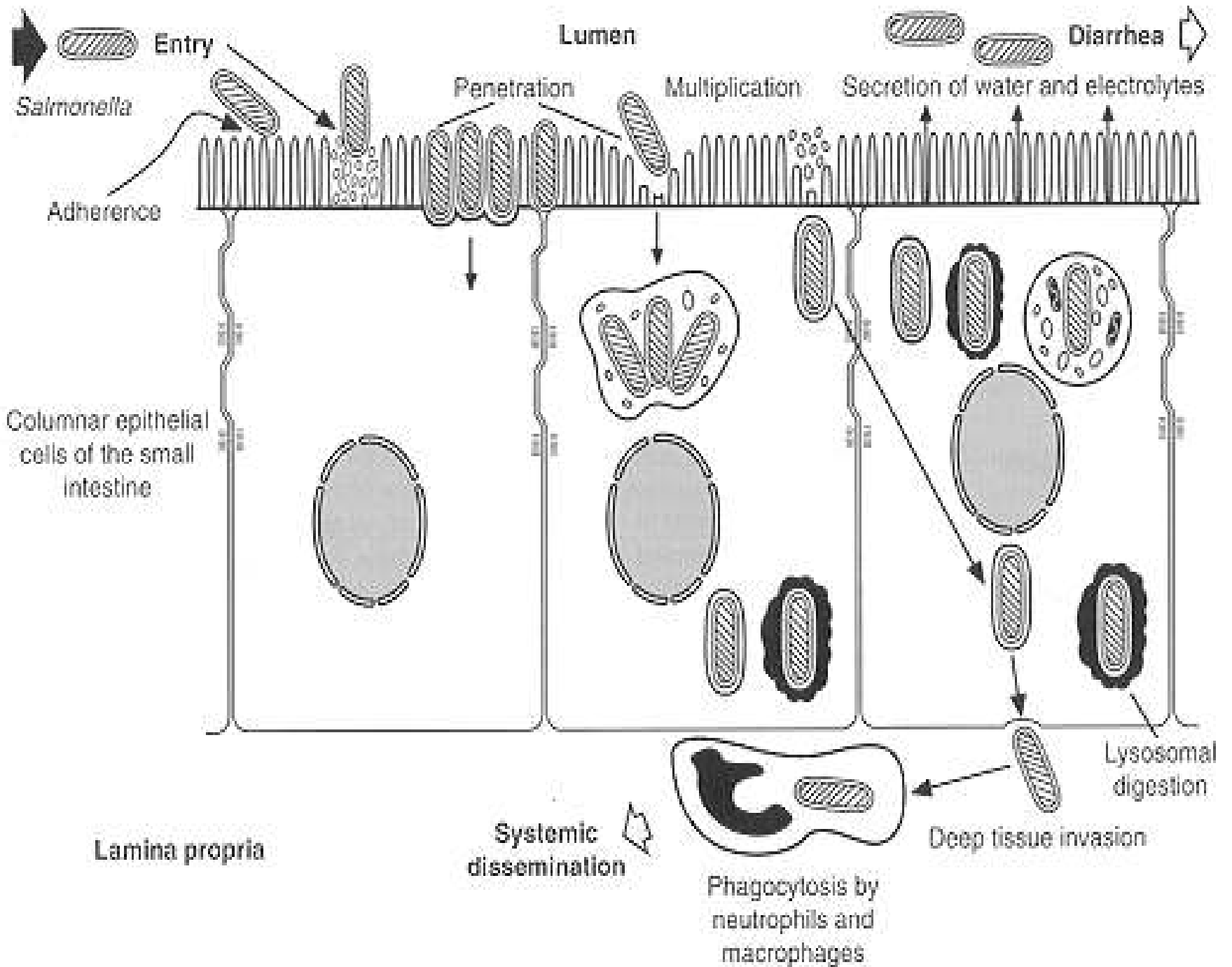


Lamina propria = thin membrane between epithelium & basement layer

Hyperplasia = abnormal increase in # of normal cells

Hypertrophy = abnormal increase in normal tissue/organ size

Prostaglandins = potent mediators of diverse set of physiologic processes



Pathogenesis of Salmonella

Enteritis (cont.)

Virulence attributable to:

- **Invasiveness**
- **Intracellular survival & multiplication**
- **Endotoxin**
- **Exotoxins: Effects in host have not been identified**
 - Several *Salmonella* serotypes produce enterotoxins similar to both the heat-labile (LT) and heat-stable enterotoxins (ST), but their effect has not been identified
 - A distinct cytotoxin is also produced and may be involved in invasion and cell destruction

Pathogenesis of Salmonella (cont.)

Invasiveness in Enteritis (cont.)

- Penetrate mucus, **adhere to and invade into epithelial layer** (enterocytes) **of terminal small intestine** and further into subepithelial tissue
- Bacterial cells are **internalized in endocytic vacuoles** (intracellular) and the organisms **multiply**
- PMN's confine infection to gastrointestinal (GI) tract, but organisms **may spread hematogenously** (through blood, i.e., septicemia) to other body sites
- **Inflammatory response** mediates release of prostaglandins, **stimulating cAMP and active fluid secretion** with loose **diarrheal stools**; **epithelial destruction occurs during late stage of disease**

Epidemiology & Clinical Syndromes (cont.)

Enteric Fevers

- ***S. typhi*** causes **typhoid fever**
- S. paratyphi A, B*** (*S. schottmuelleri*) and **C** (*S. hirschfeldii*) cause milder form of enteric fever
- **Infectious dose = 10^6 CFU**
- **Fecal-oral route of transmission**
 - Person-to-person spread by chronic carrier
 - Fecally-contaminated food or water
- **10-14 day incubation period**
- Initially signs of **sepsis/bacteremia** with **sustained fever** (delirium) for \geq one week before abdominal pain and **gastrointestinal symptoms**

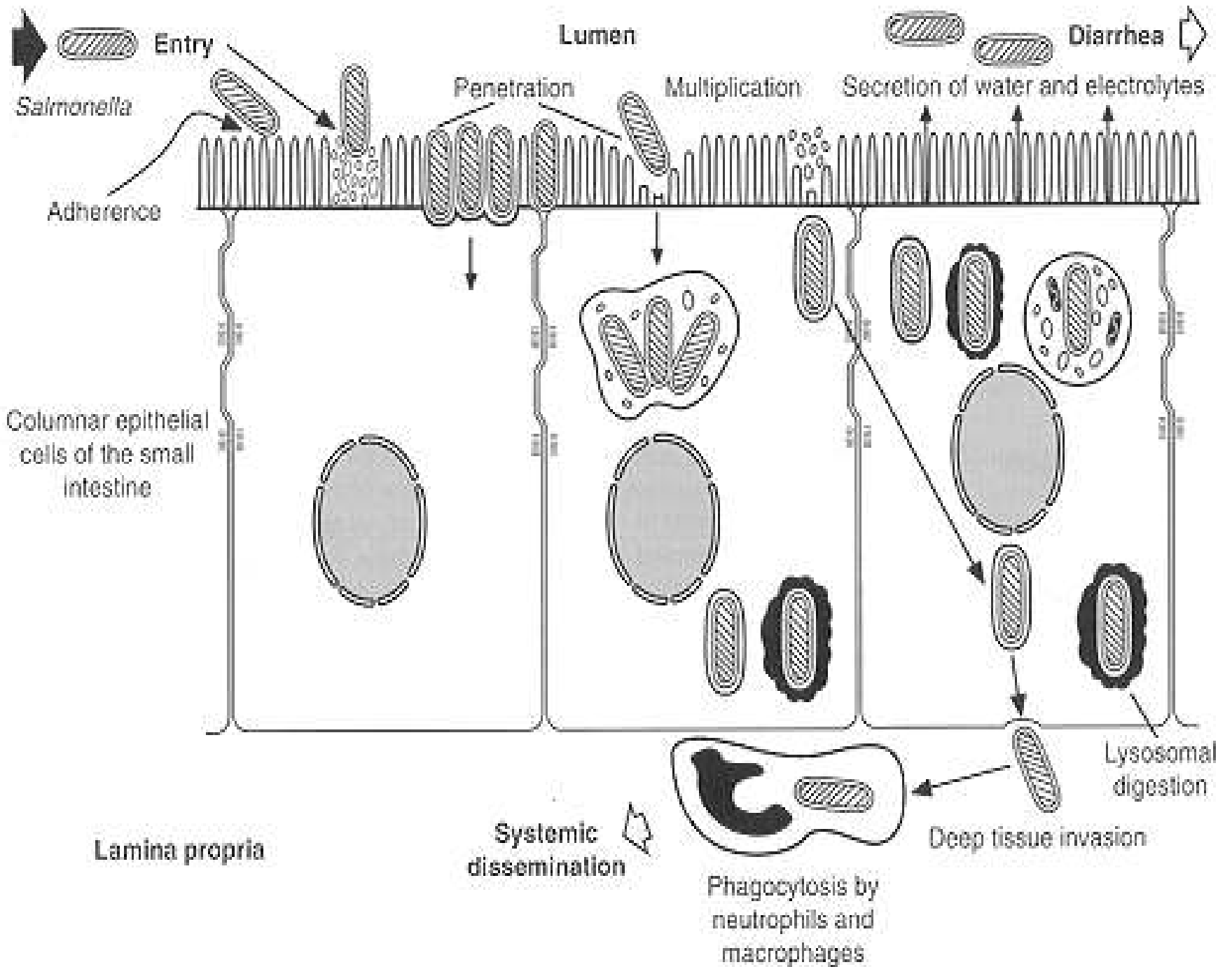
Pathogenesis of Salmonella (cont.)

Enteric Fevers (cont.)

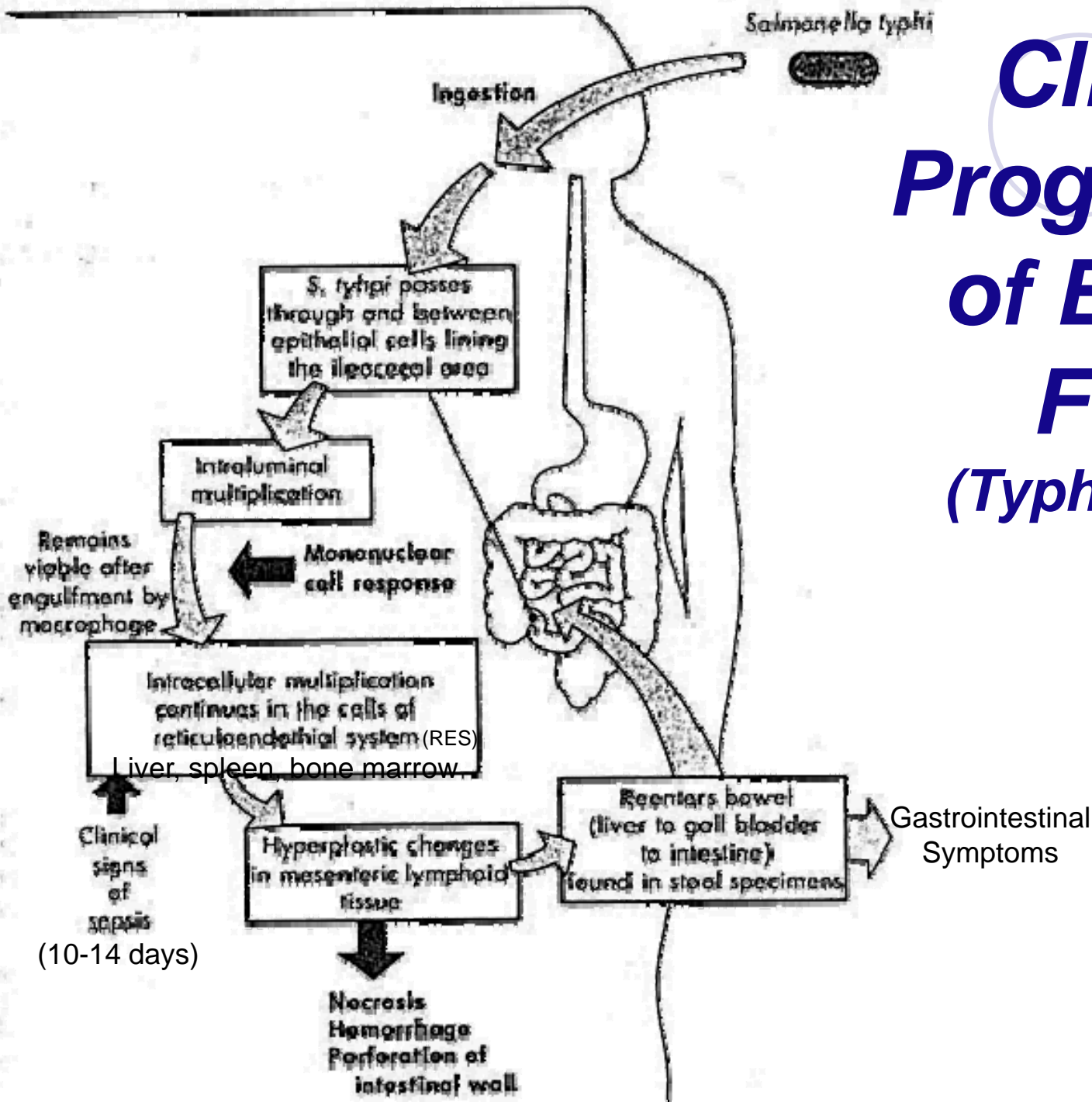
Virulence attributable to:

➤ Invasiveness

- **Pass through intestinal epithelial cells** in ileocecal region, infect the **regional lymphatic system**, invade the **bloodstream**, and infect other parts of the reticuloendothelial system
- Organisms are **phagocytosed** by macrophages and monocytes, **but survive, multiply** and are **transported** to the liver, spleen, and bone marrow where they **continue to replicate**
- **Second week:** organisms **reenter bloodstream** and cause **prolonged bacteremia**; biliary tree and other organs are infected; gradually increasing **sustained fever** likely from endotoxemia
- **Second to third week:** bacteria colonize **gallbladder**, **reinfect intestinal tract** with **diarrheal symptoms** and possible necrosis of the Peyer's patches



Clinical Progression of Enteric Fever (Typhoid fever)



Lumen (intraluminal); ileocecal area = see above - *Anatomy of Digestive Tract*

RES = sum total of strongly phagocytic cells; primarily found in lymph nodes, blood, liver, spleen and bone marrow

Hyperplastic changes = see *hyperplasia* above - *Clinical Progression of Enteritis*

Epidemiology & Clinical Syndromes (cont.)

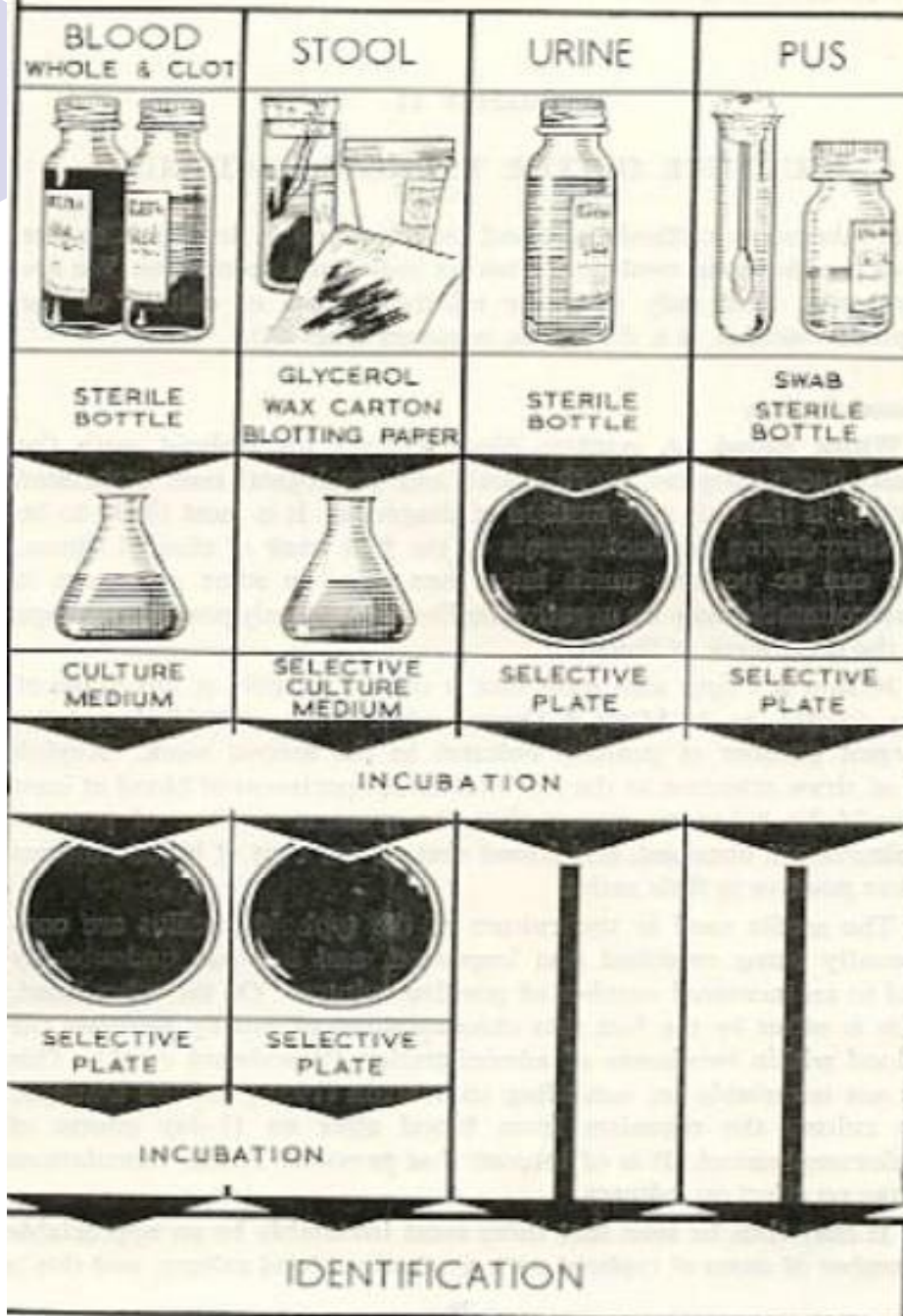
Septicemia

- Can be caused by all species, but **more commonly associated with *S. choleraesuis*, *S. paratyphi*, *S. typhi*, and *S. dublin***
- **Old, young and immunocompromised** (e.g., AIDS patients) at increased risk

STAGE	EXAMINATION	RESULT (%)
1st week	Blood culture	90%
	Blood picture	Leucopenia with relative lymphocytosis
2nd week	Blood culture	75%
	Widal test	Low titre antibody

3rd week	Widal test	100%
	Blood culture	60%
	Stool & Urine culture	80%
4th week	Widal test	100%
	Stool, Urine culture	90%
	Blood culture	25%

CULTURE OF THE TYPHOID BACILLUS



Blood culture



- Specimen: 5-10 ml. blood by venepuncture is transferred directly into a blood culture bottle containing 50-100 ml 0.5% bile broth or glucose broth.
- Liquid (Sodium polyanethol Sulphonate) may be added in the media which counteracts the bactericidal action of blood
- Incubation overnight at 37⁰ C

Blood culture :



Blood culture (cont.)

- Subculture on Mac Conkey agar or DCA
 - Further follow up is to be done
 - Castaneda's method of blood culture: By using diphasic medium, broth has an agar slant on one side.
- Provides both liquid (liver infusion broth) and solid media (3% nutrient agar slope) in one bottle.

Clot culture



- 5 ml of blood is withdrawn from the patient into a sterile test tube & allowed to clot.
- Serum used for Widal test
- Clot is broken up with sterile glass rod & added to a bottle of bile broth.
- Lysis of the clot is done by adding Streptokinase (100 units / ml)
- Adv: Higher rate of isolation than blood culture

Widal test

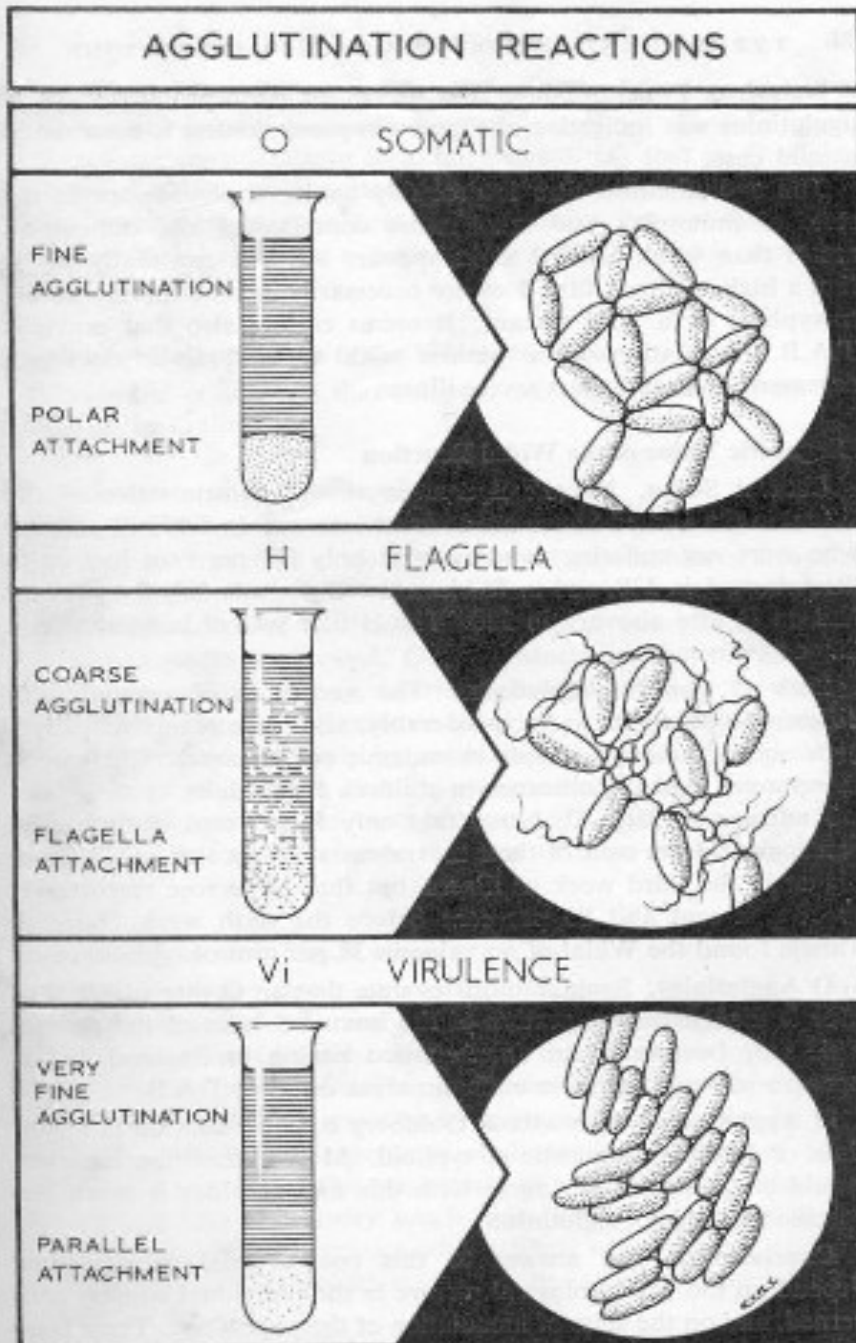


- Agglutination test which detects presence of serum agglutinins (H and O) in patient's serum
- Starts appearing in serum at the end of first week and rises sharply during third week of enteric fever
- Two specimens of sera at an interval of 7-10 days to demonstrate a rising antibody titre

Interpretation

A decorative graphic at the top of the slide consists of two groups of three circles. The first group on the left has a solid light purple circle on the left, a white circle with a light purple outline in the middle, and a solid light purple circle on the right. The second group on the right has a solid light purple circle on the left, a white circle with a light purple outline in the middle, and a solid light purple circle on the right.

- H agglutination: Loose and cotton woolly clumps
- O agglutination: Granular deposit at the bottom
- Maximum dilution of serum at which agglutination occurs indicate the titre of antibodies



O agglutination: Granular deposit at the bottom

H agglutination:

- **Loose and cotton wooly clumps**

- **Maximum dilution of serum at which agglutination occurs indicate the titre of antibodies**

Interpretation (cont.)

- Rising titre: Demonstration of rising titre of four fold or greater of both H and O agglutination at an interval of 4-7 days is the most important diagnostic criteria.
- Titre increases steadily till 3rd or 4th week
- In a single test, a titre of 100 of O or more and a titre of 200 of H agglutinins signifies presence of active infection

Interpretation (cont.)



- Local titre: Due to sub clinical infection of Salmonellosis in endemic area, low titre of agglutinins is present in the serum of normal individuals, which may cause positive reactions.
- Immunisation: with TAB vaccine, vaccinated individuals may show high titres of antibody (H antibody titre 160 or more) to each of the Salmonellae.



Interpretation

- Anamnestic reaction : Persons who had past enteric infection or who have been vaccinated may develop transient anamnestic reaction during unrelated fever like malaria, influenzae etc..
- Nonspecific antigens (fimbrial antigen) may produce false positive result.

Interpretation



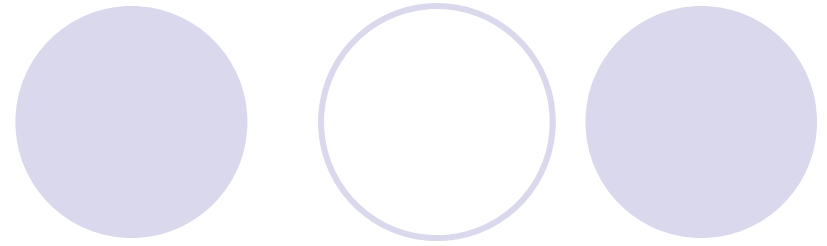
- Antibiotic treatment: When treatment with Chloramphenicol is started before the appearance of agglutinins, rising titre is not to be suspected.

Prophylaxis



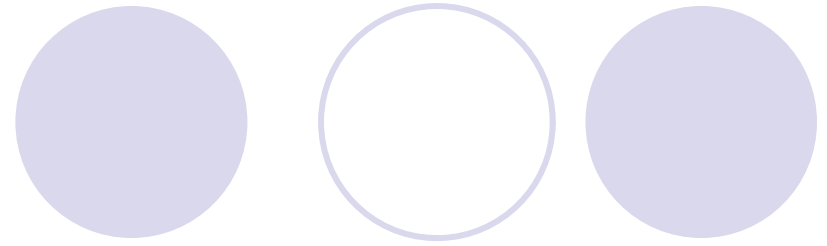
- TAB vaccine: Heat killed typhoid bacillus- Contained *S.typhi*, 1000million and *S.paratyphi* A & B, 750 million each per ml killed by heating at 50-60⁰ C and preserved in 0.5% phenol
- A divalent typhoid-paratyphoid A vaccine: Two doses of 0.5 ml. subcutaneously at interval of 4-6 wks

Prophylaxis (cont.)



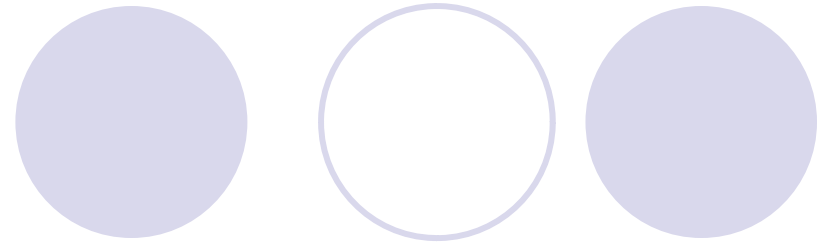
- Monovalent typhoid vaccine
- Live oral vaccine (typhoral) : Stable mutant of *S.typhi* strain Ty2 1a, lacking the enzyme UDP-galactose-4-epimerase. It is enteric coated capsule containing 10^9 viable lyophilised mutant bacilli. The course consists of one capsule orally, taken an hour before food, with a glass of water or milk, on days 1,3 & 5

Prophylaxis (cont.)

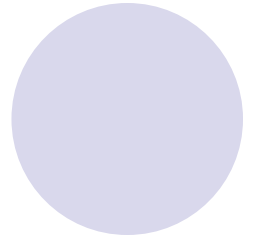
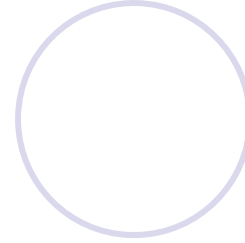
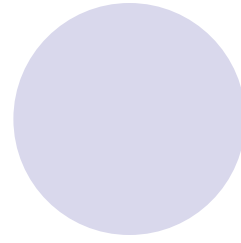
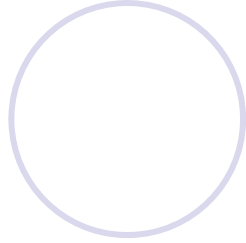
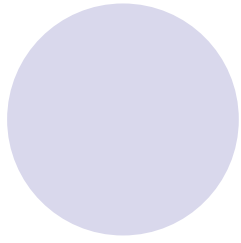


- The injectable vaccine (typhim-Vi):
Contains purified Vi polysaccharide antigen (25 μ g per dose) from *S.typhi* strain Ty2. It is a single subcutaneous or intramuscular injection which causes only minimal local reaction.

Prophylaxis (cont.)



- Recommendation:
- Only in those over five yrs. Of age
- Same dose being used for children & adults
- Protection commences 2-3 wks after administration & lasts for at least 3 years.
- Booster dose: After 3 yrs.



THANK U