Histopathological study of *Salmonella typhimurium* infection in laboratory mice by using the light and electron microscope

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Abstract:

This study was designed to evaluate the histopathological changes for internal organs of white female mice after inoculation with *Salmonella typhimurium* in dose $1x10^8$.Sixteen white mice apprrximatly age (one-two months) and body weight were (25-30) gram divided into 2 equal groups. The first group was inoculated with Salmonella typhimurium orally and killed after24hr.Group2 was inoculated with normal saline as control group. The histopathological changes of the liver were showed infiltration of kupffer cells and aggregation of mononuclear cells around the central vein with congestion of blood vessels and infiltration of inflammatory cells. The intestinal changes showed hyperplasia of goblet cells and infiltration of inflammatory cells in the lamina propria of atrophic villi. The results of the electron microscope were showed *S. typhimurium* lie close to the brush border of the villi of ileum of infected mice, and in another section noticed degeneration of the brush border and the apical cytoplasm with cavity formation occurs near a bacterium (arrow). In addition,budding, swelling and elongation of microvilli are evident.In summary to the above, the microorganisms *Salmonella typhimurium* have the ability to infect ileum and penetrate to other internal organs.

دراسة نسجية مرضية با ستخدام المجهر الضوئي و الالكتروني للإصابة بجرثومة السالمونيلا *Salmonella typhimurium* في الفئران المختبرية

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الخلاصة:

صممت هذه الدراسة لتقييم التغيرات المرضية النسجية للاعضاء الداخلية لاناث الفئران بيضاء اللون بعد حقنها بجراثيم السالمونيلا *Salmonella typhimurium* وبجرعة مقدارها (1*10 8)،تم اختيار ستة عشر فارة بيضاء اللون تراوحت اعمارها من (شهر– شهرين) واوزانها من (25–30) غرام، قسمت الى مجموعتين متساويتين المجموعة الثانية حقنت بالمحلول الملحي المتعادل كمجموعة سيطرة. اظهرت التغيرات النسجية في الكبد بارتشاح خلايا كوفر وتجمع الخلايا وحيدة النواة حول الوريد المركزي مع احتقان الاوعية الدموية وارتشاح الخلايا الالتهابية ، اما في الامعاء تميزت التغيرات فرط تنسج الخلايا الكاسية وارتشاح الخلايا الالتهابية في الصفيحة الاصيلة lamina propria للزغابات المعوية. اظهرت نتائج الفحص بالمجهر الالكتروني وجود جراثيم السالمونيلا بالقرب من حافة الفرشاة للزغابات للمعي اللفائفي للفئران المصابة ،و في مقطع اخر لوحظ تحلل حافة

الفرشاة وقمة السايتوبلازم مع حدوث تجويف بالقرب من التجمع الجرثومي، بالاضافة الى انه تم التثبت على وجود تبرعم وانتفاخ واستطالة للزغيبات، وخلاصة ما تقدم ان جراثيم السالمونيلا *S. typhimurium* لها القابلية على اصابة المعاء (اللفائفي) و اختراقه الى اعضاء الجسم الاخرى.

Introduction:

Salmonella infections are zoonotic; they can be transmitted by humans to animals and vice versa. Infection via food is also possible. *S. typhimurium*, causes a wide range of infections in birds and mammals and food poisoning in humans (1). In humans, ingestion of various *Salmonella* serovars gives rise to infection of the small intestine and to gastroenteritis. A small number of *Salmonella* serovars can lead to systemic infection and enteric fever (2). However, in mice, infection with *S. typhimurium* gives rise to enteric fever, with symptoms similar to those observed in humans after infection with *S. typhi* (2). *S. typhimurium* infection in mice is therefore widely accepted as an experimental model for typhoid fever in humans (3). *Salmonella typhimurium* is a pathogenic Gram-negative bacteria predominately found in the intestinal lumen. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharides (LPS) which protect the bacteria from the environment (4). Natural or experimental infections of animals with Salmonella result in stimulation of both humoral and cell-mediated immunity. These immune responses primarily occur against the lipopolysaccharide (LPS) and major outer membrane (OM) proteins (5). Although the innate mechanisms of the immune system are highly effective in restricting the initial growth of *S. typhimurium* for several days. *S. typhimurium* successfully adapts to the enormous pressure imposed by the innate immune system by expressing an array of virulence factors that improve its resistance to bactericidal host mechanisms. Only the generation of a specific lymphocyte response allowsthe eventual effective eradication of bacteria, and provides increased protection against subsequent encounter with this pathogen (6).

Materials and Methods:

- Tissue culture:

Salmonella typhimurium serotype was obtained from Zoonoses Unit/ Veterinary Medicine/ Baghdad University, and the biochemical properties were tested depending on the method of (7).

- Experimental Design:

16 animals (mice) were used in this experiment both males and females that divided into two groups:

1- The first group- was infected by *Salmonella typhimurium* bacteria through oral route with the dosage 1×10^8 depending on the method of (8), and the mice of this group were killed after less than 24 hours, the internal organs were taken for making the histopathological sections, and the intestine was taken

for electronmicroscopy and the test was worked in (Electronmiocr-oscopy department / Medicine college/ Nahrain University).

2- The second group- this group was injected with Normal saline as control group.

Result:

1-Histopathological lesions:

a- Liver:

After less than 24 hours showing proliferation of kupffer cells and mononuclear cells aggregation around central veins as well as congestion of the blood vessels with infiltration of inflammatory cells in the lumen (Figure a).

b- Intestine:

After less than 24 hours showing hyperplasia of goblet cells, inflammatory cells in the lamina propria of atrophic villi (Figure b)**.**

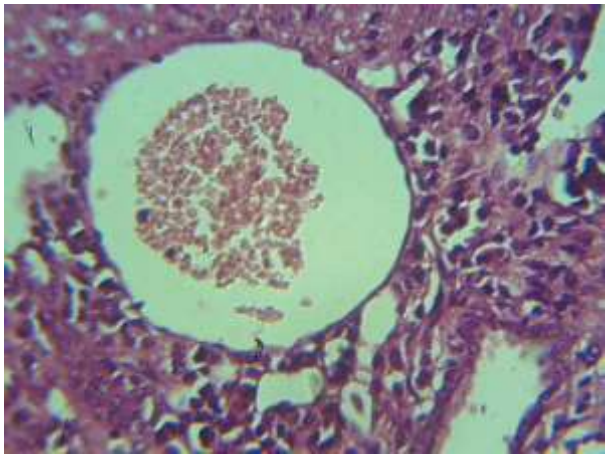

Fig. a: The microscopic section of the liver of mouse after treatment with *S. typhimurium* orally revealed proliferation of kupffer cells and mononuclear cells aggregation around central veins as well as congestion of the blood vessels with infiltration of inflammatory cells in the lumen (H&E X400).

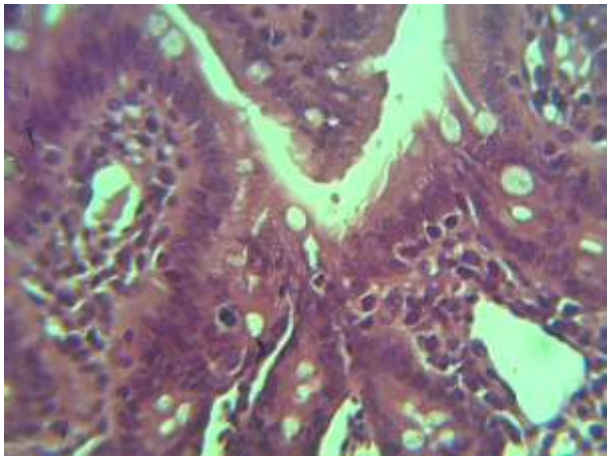

Fig. b: The histopathological lesions of the intestine of mouse after treatment with *S. typhimurium* orally characterized by hyperplasia of goblet cells, inflammatory cells in the lamina propria of atrophic villi (H&E X400).

2 -Ultrastructural findings:

The ultrastructural findings refers to several organisms lie close to the brush border which is still intact of epithelial cells at the mid-villus portion of the mouse ileum after orally treatment with *S.typhimurium* less than 24 hours and cytoplasmic components are well preserved (Fig. 1). Cells at the mid-villus portion after challenge shows degeneration of the brush border and the apical cytoplasm with cavity formation occurs near a bacterium (arrow) also budding, swelling and elongation of microvilli are evident. After challenge with *S.typhimurium* the microvilli, terminal web and apical appears at sites of bacterial penetration (arrows) and the cytoplasm are replaced by a shallow and a deep cavity in (Fig. 3), and degenerated microvilli,blebs and vesicles also a bleb containing small vesicles in(Fig. 4). Other cytoplasmic organelles and adjacent cells are unaltered.

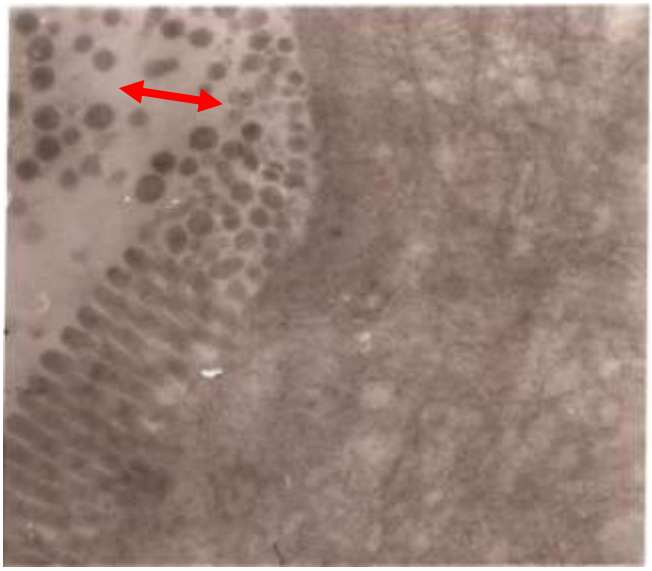

Fig (1): Electron micrographs of absorptive epithelial cells at the mid-villus portion of the mouse ileum less than 24 hours after challenge with *S. typhimurium* ,shows several organisms lie close to the brush border which is still intact ( ). Cytoplasmic components are well preserved (X 25,000).

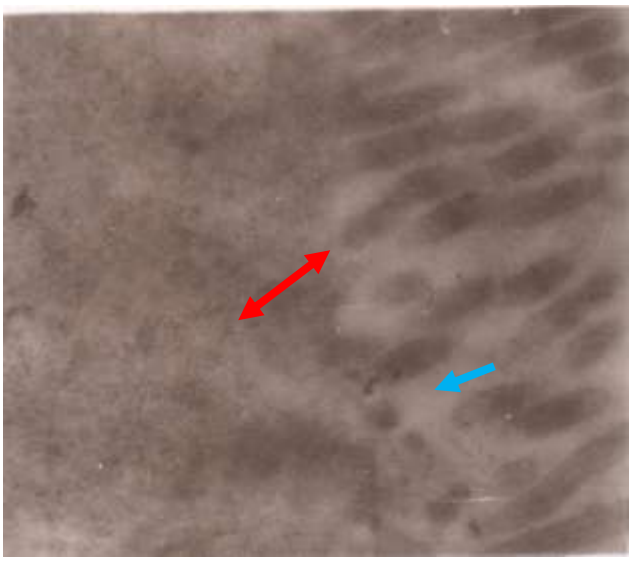

Fig (2): Electron micrographs of absorptive epithelial cells at the mid-villus portion of the mouse ileum 24 hours after challenge with *S. typhimurium*, shows degeneration of the brush border and the apical cytoplasm with cavity formation occurs near a bacterium (arrow) ( ). Budding, swelling and elongation of microvilli are evident ( ). (X 46,000)

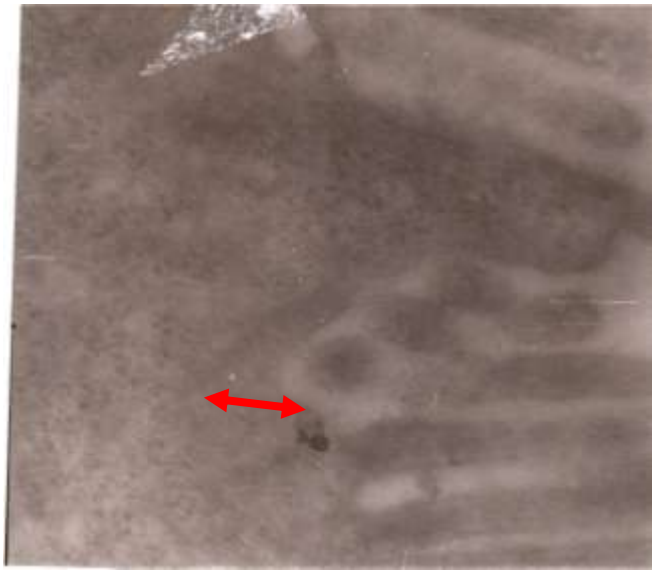

Fig (3): Electron micrographs of absorptive epithelial cells at the mid-villus portion of the mouse ileum 24 hours after challenge with *S. typhimurium* ,The microvilli, terminal web and apical appears at sites of bacterial penetration (arrows) ( ),cytoplasm are replaced by a shallow and a deep cavity. The remaining cytoplasmic organelles are intact(X 64,000).

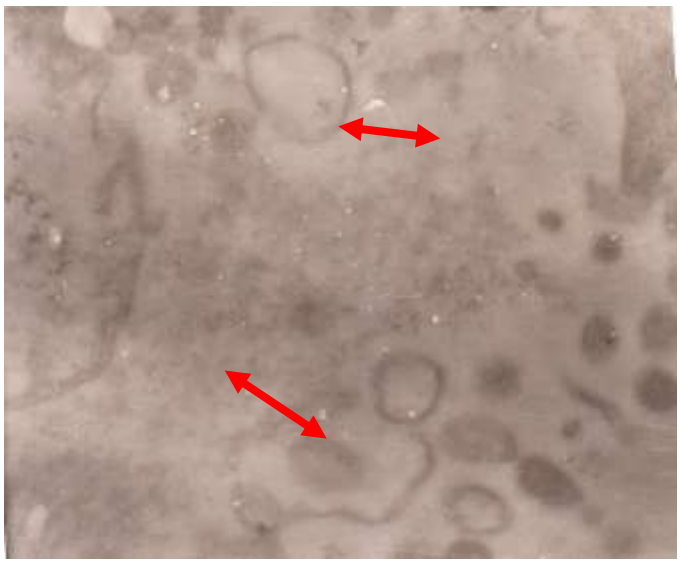

Fig (4): Electron micrographs of absorptive epithelial cells at the mid-villus portion of the mouse ileum 24 hours after challenge with *S. typhimurium* , terminal web and apical cytoplasm in which degenerated microvilli,blebs and vesicles are present ( )and a bleb containing small vesicles. Other cytoplasmic organelles and adjacent cells are unaltered(X 46,000).

Discussion:

After oral ingestion and colonization of the small intestine, Adherence to the intestinal mucosa is the first step in the establishment of persistent Salmonella colonization of the gut (9).The probability of systemic infection resulting from mucosal invasion is directly related to the number of salmonellae that initially colonize the intestinal epithelium (10). Demonstrate *Salmonella typhimurium* is a pathogenic Gram-negative bacteria predominately found in the intestinal lumen. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharides (LPS) which protect the bacteria from the environment (5). The LPS is made up of an O-antigen, a polysaccharide core, and lipid A, which connects it to the outer membrane. Lipid A is made up of two phosphorylated glucosamines which are attached to fatty acids. These phosphate groups determine bacterial toxicity. Animals carry an enzyme that specifically removes these phosphate groups in an attempt to protect themselves from these pathogens (11). The O-antigen, being on the outermost part of the LPS complex is responsible for the host immune response. *S. typhimurium* has the ability to undergo acetylation of this O-antigen, which changes its conformation, and makes it difficult for antibodies to recognize (12). lesions of the intestine after treatment with *S. typhimurium* orally characterized by hyperplasia of goblet cells, inflammatory cells in the lamina propria of atrophic villi in (Fig. b), and the result agree with (13) that said *Salmonella typhimurium* causes gastroenteritis in humans and other mammals. When the bacterial cells enter epithelial cells lining the intestine they cause host cell ruffling which temporarily damages the microvilli on the surface of the cell. This causes a rush of white blood cells into the mucosa, which throws off the ratios between absorption and secretion.

Salmonella infection of the intestinal tract results in damage to the gut epithelium. Intestinal segments infected with *Salmonella typhimurium* had high levels of fluid secretion as early as 6 h post-bacterial infection. At 20 h post-infection, high levels of TNF activity were present in fluids obtained from infected intestinal segments (14). And this may explain the hyperplasia of goblet cells. *S. typhimurium* penetrates the intestinal epithelium and enters the Peyer's patches, lymphoid structures that line the intestine (15). (Fig. a) showed by the microscopic section proliferation of kupffer cells and mononuclear cells aggregation around central veins as well as congestion of the blood vessels with infiltration of inflammatory cells in the lumen of the liver after treatment with *S. typhimurium*, and this result agree with (16) that said *S. typhimurium*, the main entrance into the Peyer's patches appears to be M cells, a

specialized cell population overlaying the Peyer's patches and involved in antigen sampling from the intestinal lumen into these lymphoid follicles. From the Peyer's patches, *S. typhimurium* moves into the mesenteric lymph nodes, and from there bacteria spread via the efferent lymph to the circulatory system, leading to transient bacteremia (17). Bacteria are rapidly cleared from the blood by phagocytes in spleen and liver, and a large fraction of bacteria are killed by these cells (18). These first stages of *Salmonella* infection, which are normally completed within a few hours, as a consequence, secondary bacteremia, endotoxic shock, and rapid death ensue (17). In contrast, during non-fatal infection, mice restrict bacterial titers at a certain level. The subsequent phase of infection is characterized by splenomegaly. This study has demonstrated that *S. typhimurium is* capable of penetrating through the intercellular tight junction. (Fig. 1,2). At 24 hours, the number of bacteria engulfed by the phagocytes present in the mucosal lining. And this result agrees with (8) that said the brush border of the small intestine is an important region of infection. The approach of a single pathogen into critical proximity to the microvilli triggers sudden local degeneration of the brush border. After penetration, the effect of the organisms upon the host cell is localized. Alterations such as budding and fusion of microvilli are not specific features of Salmonella infection, "6 suggesting that it is not an abnormal condition. These changes in microvilli may also be produced artificially by fixing intestinal mucosa in a hypotonic solution.17 flexneri 2a, only the virulent strain invades the mucosal barrier and multiplies in the lamina propria; the avirulent strain is unable to penetrate the epithelium (8).

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