A STUDY ON THE BACTERIAL DISSEMINATION AND EXPERIMENTAL PATHOLOGY OF *SALMONELLA PARATYPHI* – AN INFECTION IN WHITE MICE

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Abstract

Background: Paratyphoid infection remains an important public health problem with marked host specificity for humans or higher primates but not naturally virulent for mice except that using high inoculum of the paratyphoid bacilli.

Objective:

1. Study the bacterial dissemination through the organs of white mice.
2. Study the pathological changes associated with this experimental disease process

Methods: One LD50 of the microorganism corresponded to $8x10^7$ bacterial Cell/ml. However 10LD50 doses were intraperitoneally used for mice to produce extensive disease process in mice. Following inoculation of mice the course bacterial dissemination and pathological lesions were studied at specific intervals and for 3 days post inoculation.

Result: The microorganisms were persistent in the spleen and liver for 21 and 17 days postinoculation respectively, where as the microorganisms persisted in the mediastinal lymph nodes and lungs for 9 days and kidney and heart blood for 5 days postinoculation. The main pathological lesions were initiated as a mild infiltration of neutrophils and edema in the spleen liver and lymph node the neutrophils iflteration will be gradully replaced by mononuclear cell infiltration and finally with fibroblasts proliferation.

Conclusion: The Salmonella paratyphi-A is of lower pathogenicity and virulence for white mice, through their dissemination in organs of white mice and associated pathological findings.

Keywords: Salmonella paratyphi-A infection white mice

IRAQI J MED SCI, 2007;VOL.5(3):23-30

Introduction:

Paratyphoid bacilli are enteropathogenic bacteria with marked host specificity, *Salmonella paratyphi* type A, B & C is strictly pathogenic for humans (or higher primates) but not naturally virulent for mice. To kill a mouse, it is necessary to give high inoculum intraperotineally, death occurs within 1-4 days following the development of a toxic syndrome (1). In our country as in other parts of the developing countries, febrile illness due to typhoid and paratyphoid bacilli are common. Although the clinical picture, the epidemiological picture as well as diagnostic procedures of paratyphoid fever have been well studied, some aspects related to the role of bacteria in the disease process and the pathological changes associated with the disease still require some illumination. Thus, the objective of this study was to fulfill a required hypothesis that paratyphoid infection can be simulated in a suitable laboratory animal model that provides a picture for human paratyphoid, the present study aims at the followings:

1- Study the disease process, including the bacterial dissemination through the organs of the white mice

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Received: 6th June 2000, Accepted: 21st December 2005.

experimentally infected with *Salmonella paratyphi* - A.

2- Study the pathological changes associated with this experimental disease process in the white mice.

Materials and methods:

White mice, weighing 15-20 Gms, 2 months old, were obtained from Al-Kindi Company for Veterinary drugs and vaccines production. The mice were healthy and reared on concentrated food for two weeks before being used. A local strain of *Salmonella paratyphi* - A, isolated from the febrile patients, in the Ibn-Khatib hospital and their LD50 dose corresponded to $8x10^7$ bacterial cell. A logarithmic phase growth of *Salmonella paratyphi* - A in trypticase soy broth at 37ºc was taken, washed once in phosphate buffer saline, a suspension of a viable count of 10^9 bacterial cell/ml was obtained. Fifty mice were intraperitoneally injected with 0.8 ml of *Salmonella paratyphi* - A suspension containing $8x10^8$ bacterial cells (10 LD50). Two inoculated mice were sacrificed every two days including the dead mice for the period of one month. All the sacrificed and dead mice were studied for the purpose of:

1- Isolation of *Salmonella paratyphi* - A from the different organs of the experimentally infected mice, looking for bacterial dissemination in the organs.

2- For pathological study, small representative pieces from all the organs of infected mice were fixed in 10% neutral buffered formalin, processed routinely, cut at 5-µ thicknesses and stained with hematoxylin and eosin (H&E).

Results:

1- Distribution of *Salmonella paratyphi* - A in the organs of experimentally infected white mice:

During 30 days of experimental infection of mice with *Salmonella paratyphi* - A, an extensive dissemination of this microbe was found in the different organs at different intervals post infection (PI). It is evident (Table – 1) that the spleen and liver found to be the main target organs of invasion, whereas, kidneys and lungs were slightly invaded by this microbe. The spleen had the longest period of infectivity, which lasted for 21 days post infection, whereas, infectivity lasted for 17 days in the liver. Mediastinal lymph nodes and lungs harbored the organism for 9 days. The organisms were isolated from the kidneys and heart blood for 5 days post infection. The brain is free of bacterial isolation.

Table –1: Distribution of *Salmonella paratyphi* - A through the organs of white mice after intraperitoneal inoculation

Intervals (PI) *	Spleen	Liver	Lungs	Media-stinal lymph node	kidneys	Heart blood	Brain
1st day	+	+	+	+	+	+	-
3rd day	+	+	+	+	+	+	-
5th day	+	+	+	+	+	+	-
7th day	+	+	+	+	-	-	-

9th day	+	+	+	+	-	-	-
11th day	+	+	-	-	-	-	-
13th day	+	+	-	-	-	-	-
15th day	+	+	-	-	-	-	-
17th day	+	+	-	-	-	-	-
19th day	+	-	-	-	-	-	-
21st day	+	-	-	-	-	-	-
23rd day	-	-	-	-	-	-	-
25th day	-	-	-	-	-	-	-
27th day	-	-	-	-	-	-	-
29th day	-	-	-	-	-	-	-

*** PI: Post infection.**
**** Two mice were sacrificed for each interval**.

2- The Pathological Findings:

Different pathological findings appeared in this experimental disease processes were as follows:

The spleen:

The earliest lesions were mild infiltration of neutrophils in white and red pulps of the splenic tissue (Fig.-1). These inflammatory cells were gradually replaced by mononuclear cells (lymphocytes and macrophages), and few fibroblasts through the second and third week post infection. Also there is extensive congestion and hyperplasia of white pulp and in the reticuloendothelial cells lining the red pulp. These lesions were completely disappeared through the fourth week postinfection.

The liver:

It showed initially a mild aggregate of neutrophils in the sinusoidal and preisinusoidal areas of the hepatic tissue (Fig.-2), which is extensively congested and these inflammatory cell infiltrations were gradually replaced with few mononuclear cells (Lymphocytes and macrophages) (Fig.-3), through the second week post infection. These lesions were completely disappeared through the third week post infection.

Mediastinal Lymph Node:

There is extensive hyperplasia in the lymphoid follicles of the cortical region and in the reticuloendothelial cells lining the medullary sinuses (Fig.-4). Also there is extensive congestion of the lymphoid tissue.

The lungs:

It shows extensive congestion and mild interstitial pneumonic lesions (Fig.-5).

The Kidneys:

It showed only congestion and mild microthrombi in the renal tissue (Fig.-6).

The brain and other organs showed only congestion.

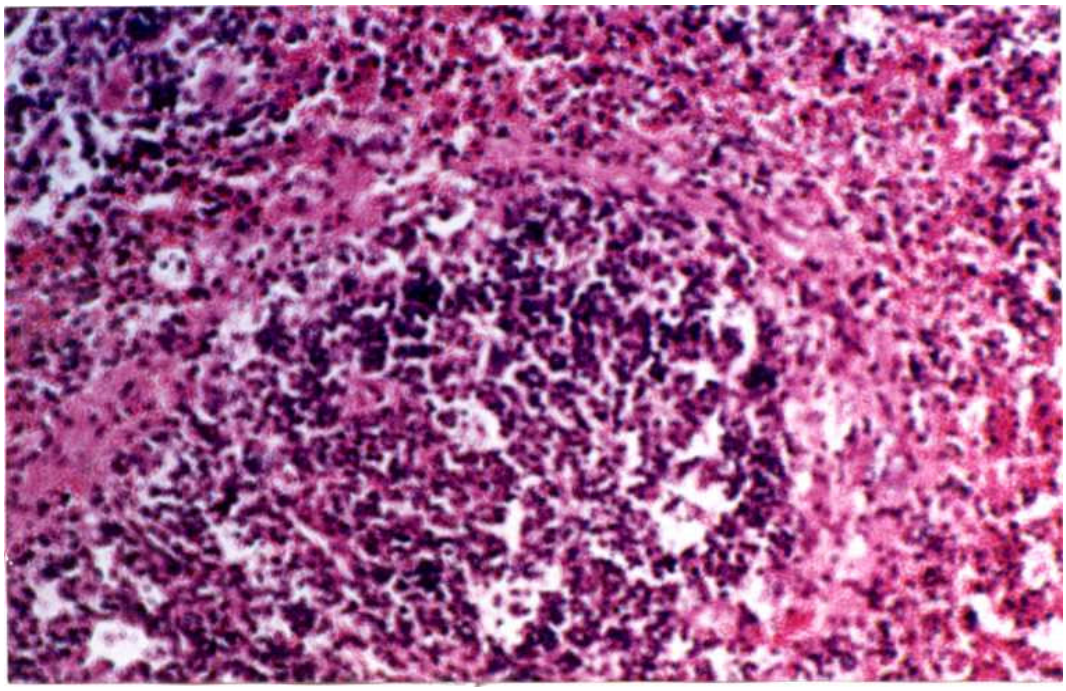

Fig.1: Microscopic section of spleen tissue shows focal aggregate of neutrophils and edema in the white pulp (H&E) X 125.

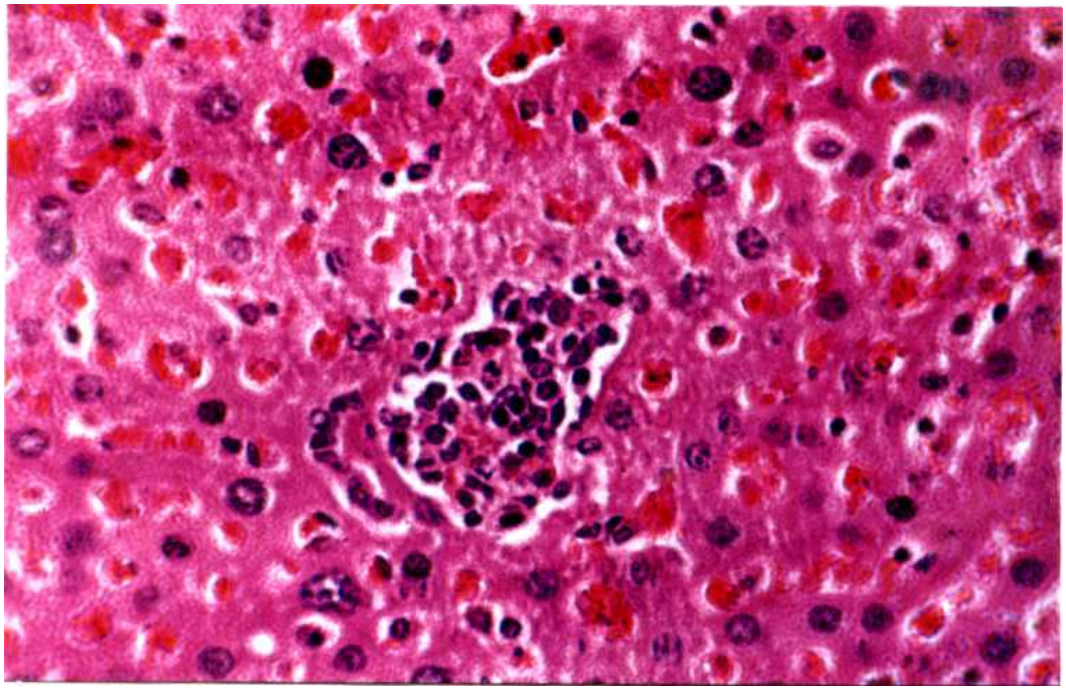

Fig.2: Microscopic section of liver tissue shows the presence of focal aggregate of neutrophils in the area adjacent to the sinusoids (H&E) X 250.

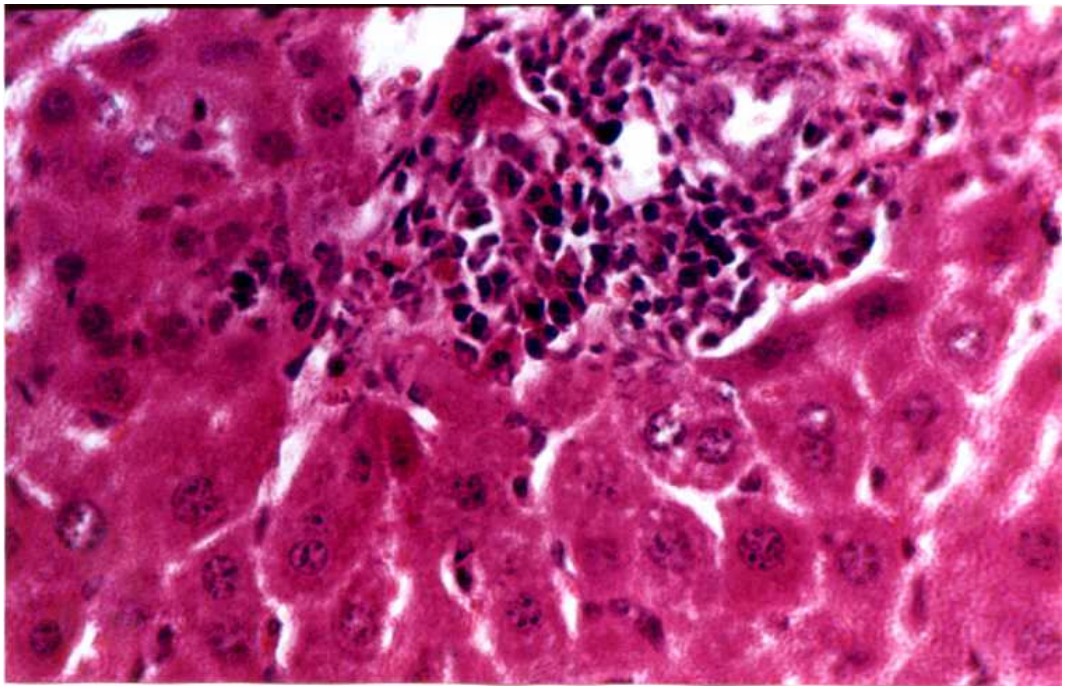

Fig. 3: Microscopic section of liver tissue shows infiltration of lymphocytes and macrophages replacing the neutrophils (H&E) X 500.

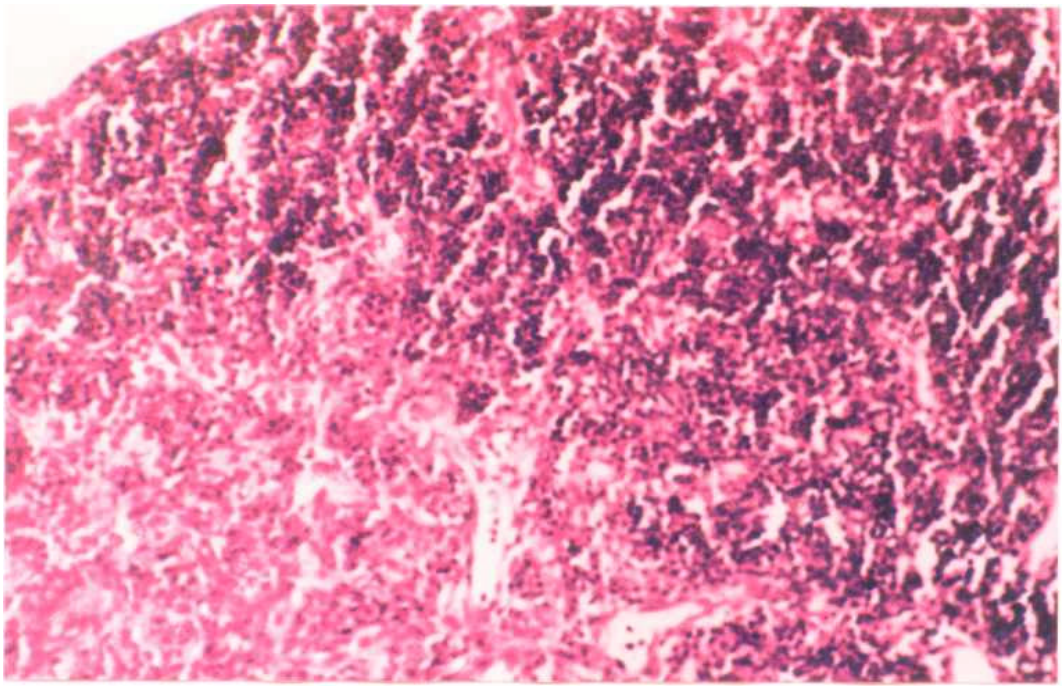

Fig. 4: Microscopic section of mediastinal lymph node shows extensive reactive hyperplasia (H&E). X 125

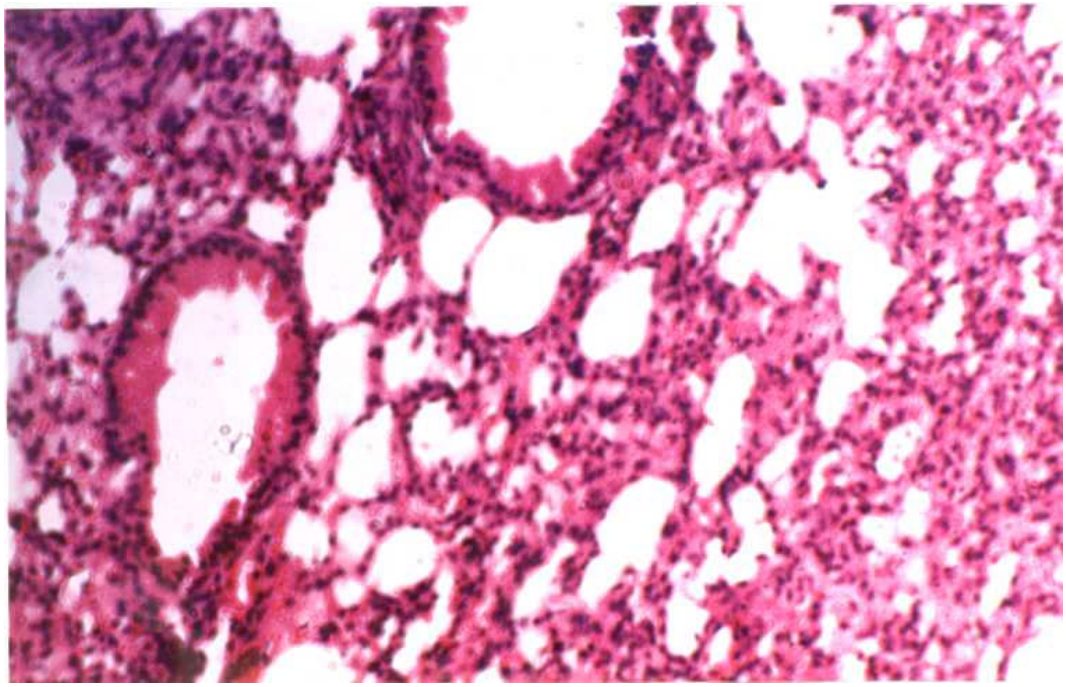

Fig. 5: Microscopic section of pulmonary tissue shows interstitial pneumonic lesions (H&E) X 125.

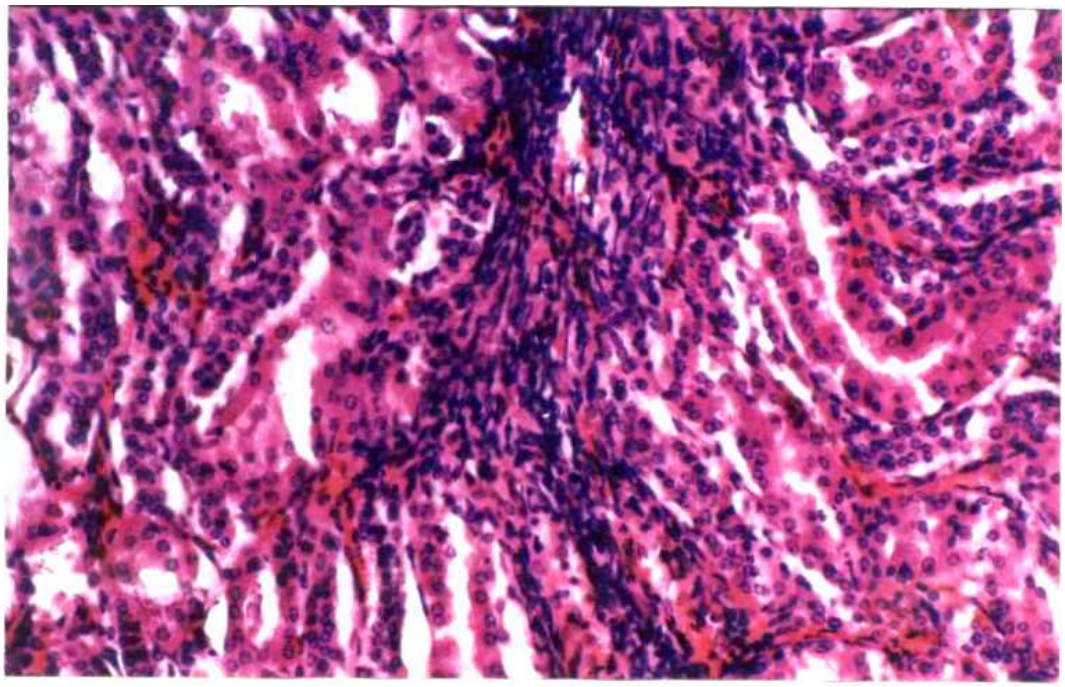

Fig. 6: Microscopic section of renal tissue, shows mild interstitial nephritis (H&E) X125

Discussion:

1- Distribution of *Salmonella paratyphi* - A in the organs of experimentally infected white mice:

Most studies on the typhoid and paratyphoid bacilli in mice have been limited by the fact that these organisms have low pathogencity for this animal species. Thus microorganisms are strictly pathogenic for human being (or higher primates). To kill mice a high

inoculum of these microorganisms should be intraperitoneally given (1). These findings were confirmed by this work, a less extent disease process produced in mice when the inoculum introduced into the peritoneal cavity was $8x10^8$ bacterial cell of *Salmonella paratyphi* - A. Corresponding results were obtained by other workers, who showed that typhoid and paratyphoid like diseases were produced in mice using the paratyphoid bacilli type-B (2) and typhoid bacilli (3, 4 and 5). This study revealed that following the intraperitoneal injection of *Salmonella paratyphi* – A with the massive dose of $8x10^8$ bacterial cell, a less extent growth of this microorganism occurred in the spleen, liver, mediastinal lymph node, lungs and kidneys; the microorganisms reach in to the thoracic duct and to blood through 24 hours post infection. These results go along with those reported by Gerichter, Carter and Collins and AL-Joboury (6, 7, 2), who isolated similar organisms (*Salmonella paratyphi* – A and B) from these organs one day post infection. The difference in the dissemination of this microbe in the different organs of the mice was attributed to the difference in the doses, route of infection and virulence of the microorganism. In this study we used $8x10^8$ bacterial cell injected intraperitoneally, whereas, other studies used 5x109, orally (6), $2x10^6$ intravenously (7) and $5x10^8$ intraperitoneally (2). In the present study following intraperitoneal injection of *Salmonella paratyphi* – A in to the mice, less extent persistence systemic growth occurred in most of the infected mice which harbored the infection in the spleen for 21 days, the liver for 17 days, in the mediastinal lymph node and lungs for 9 days and heart blood and kidneys for 5 days post inoculation, these findings are similar to those reported by Gerichter, Carter and Collins and AL-Joboury (6,7,5 and 2) for typhoid and paratyphoid bacilli.

Pathological Findings:

This study revealed mild lesions, initiated in the liver, spleen and mediastinal lymph nodes and consisted of mild infiltration of neutrophils, edema and congestion of blood vessels. These inflammatory cells infiltrations and edema were demonstrated by other workers (8 & 9) on murine Salmonellosis caused by *Salmonella typhimurium* and in mice infected with typhoid and paratyphoid bacilli (10, 2). Both workers demonstrated extensive neutrophil infiltration forming multifocal microabscesses in different organs, which is not observed in the present study and may be explained on the basis of virulence of the microbe used in the present study. During the second week post infection, the neutrophiles infiltration was gradually replaced by mononuclear cells (Lymphocytes and macrophages and plasma cells) infiltration without granulomatous type lesions which demonstrated by Naconeczna and Hsu (8, 9) on murine Salmonellosis caused by *Salmonella typhimurium* and AL-Joboury (10,2) on mice experimentally infected with typhoid and paratyphoid bacilli. The absence of granulomatous lesions in the spleen and liver in the present study may be explained on the basis of low virulence of the microorganism used in the present study, and therefore, during third and fourth week post infection, most of the infected organs will become free from any lesions. The healing of the lesions through these periods of post infection will indicate that the lesions were originally found at less extent, so easily healed. Other mild lesions such as, hyperplasia of lymphoid tissues, interstitial pneumonic lesions in the lung tissue and micro thrombi in the kidney were demonstrated in the

present study. The occurrence of these lesions in these organs were explained as metastatic type lesions occurred by the microorganisms through, the hematogenous dissemination, such metastatic lesions were also reported in mice experimentally infected by typhoid and paratyphoid bacilli (2, 10) and in typhoid patients (11,12). No brain involvement by *Salmonella paratyphi* – A, is reported in the present study, these findings may be explained on the basis of low virulence of this microbe which did not reach into the brain through the metastasis.

Conclusions:

The results of this study on mice experimentally infected with *Salmonella paratyphi* –A differ from the results of the previous study obtained by AL-Joboury (2) on mice experimentally infected with *Salmonella paratyphi* – B in the followings-:

1- The present study showed focal aggregate of neutrophils without microabscess formation and not accompanied by granulomatous lesion in spleen and liver, comparable to the focal microabscess and granuloma seen in the spleen and liver in the previous study on mice experimentally infected with *Salmonella paratyphi* – B.

2- No involvement of the brain tissue of the mice by the *Salmonella paratyphi* – A in the present study.

3- Mild nonspecific pathological lesions were seen in the lungs and kidneys of the mice in the present study.

4- The above findings indicate that the *Salmonella paratyphi* – A is of lower pathogencity and virulence than of *Salmonella paratyphi* – B and for *Salmonella typhi*.

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