

**ULTRASTRUCTURAL CHANGES OF THE
MESENTRIC LYMPH NODES OF CALVES
EXPERIMENTALLY INFECTED WITH
SALMONELLA TYPHIMURIUM**

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SUMMARY

A systemic study was done on the pathogenesis of experimentally induced Salmonella typhimurium infection in calves. The present investigation was carried out on sixteen normal colostrum fed friesian calves, ranging in age from 3 to 6 weeks. The calves were divided into two equal groups. Group I inoculated orally with (1.5×10^{11}) Salmonella typhimurium and group IA served as control.

The early ultrastructural alteration in the mesenteric lymph nodes was the presence of many free Salmonella in localized vacuoles. The interaction between the host cells and phagocytized Salmonella was also observed.

INTRODUCTION

Previously, several studies of experimental Salmonellosis have been done in animals and the result indicates that when a

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virulent strain of Salmonella typhimurium was injected into an animal, the organisms reproduced in phagocytes residing in the reticuloendothelial systems e.g. liver, spleen, lymph nodes, causing a systemic infection and producing specific lesions (typhoma) in such organs.

In a study which has been done by Akazaki *et al.* (1), found that a lesion in the lymph nodes consists of reticuloendothelial cells followed by proliferation of reticular cells in the lymphoid sinuses as well as in the parenchyma. Bohme (2), reported that the pathological changes in the lymph nodes were remarkable when compared with those in liver and spleen.

Since no work yet have been done to describe the ultrastructural changes in the mesenteric lymph nodes of calves experimentally infected with Salmonella typhimurium our present study was done to investigate the ultrastructural changes in the mesenteric lymph node at different interval periods.

MATERIALS AND METHODS

Experimental Animals

Sixteen, male, friesian calves, born and reared on a dairy farm (Arab Iraqi Company for livestock development) have been used. The dairy farm was selected because it had no history of Salmonellosis. The calves age ranged between three and six weeks and had 35-40 Kg. body weight at the time of inoculation. They were assigned at random to two groups of eight calves each.

Culture Preparation

A highly virulent and multiple drug resistant Salmonella typhimurium was used to reproduce the diseases experimentally. The strain was grown on nutrient agar slant, inoculated in trypticase soy broth and inoculated at 37 °C for 18 hours. Each calf was dosed orally through a short rubber tube with 50 ml trypticase soy broth in which the number of viable organisms in the inoculum was 1.5×10^{11} (JCFU) as estimated by the method of miles (3). Calves served as a control were given 50 ml of sterile trypticase soy broth orally.

Electron Microscopic Technique :

One calf from each experimental group was killed and necropsied in a conventional manner at each of the following periods 8, 16, 24, 36, 48, 72, 96, and 120 hours post infection (PI).

The abdomen was quickly opened, then for this study mesenteric lymph nodes was removed as quickly as possible and put on a piece of dental wax to a drop of primary fixative (0.75 % glutaraldehyde). The MLNs were dissected into cubes not greater than 1 mm³ using a new razor blade each time.

The specimens were fixed with 0.75 % glutaraldehyde in 0.1 M phosphate buffer for two hours at room temperature (4). After that they were washed with the same buffer solution several time and left overnight at 4 °C. The blocks were post fixed with 1 % Osmium tetroxide in 0.1 M phosphate buffer for one hour at 4 °C and were block stained with 1 % Uranyl acetate for two hours. After dehydration with graded ethanol of 25 % - 99 % and three changes of 100 % ethanol, two changes of propylene oxide, of which a block each was embedded in epoxy resin and polymerized by incubation at 60 °C in an oven

for 48 hours. Ultrathin section were made with an ultramicrotome and double stained with uranyl acetate and lead citrate (5). The specimens were observed with JEOL, (JEM - 1200 Ex) transmission electron microscope.

RESULTS

The ultrastructural examination of MLNs by TEM in calves infected with Salmonella typhimurium at 6 hours indicated that many Salmonella were present free in the lymphoid nodules with localized vacuoles surrounding the Salmonella. Salmonella engulfed by phagocytic cells were not detected.

At 16^o hours (PI), bacteria were present mainly in macrophages were surrounded by double layered structure of the plasma membrane, in which the Salmonella cell was swollen and its granules were diffusely distributed (Fig. 1).

By 36 hours after challenge exposure most phagocytized Salmonella were localized in area of cytoplasmic vacuoles inside the host cells particularly proliferative reticular cells, with disappearance of organelles (Fig. 2).

As the infection became more progressive, many proliferative reticular cells underwent degenerative changes characterized by vacuolation of cytoplasm, swollen mitochondria and disappearance of both cytoplasmic and nuclear matrix. Some phagocytized Salmonella in the phagosomes of macrophages were surrounded by several imoraneous structures. Various cytoplasmic vacuoles were seen and some of them contained myeloid figures (Fig. 3). Some degenerated cells contained complex configurations of membranes usually in the form of whorl surrounding the degeneration cellular components inside the mitochondria of reticular cell and macrophages (Fig 4 & 5)

DISCUSSION

The early ultrastructural changes was the presence of many free Salmonella in localized vacuoles. The interaction between the host cells and phagocytized Salmonella has the following prominent features. The phagocytized Salmonella was observed at 16 hours (PI). Salmonella were enclosed by a limiting membrane known as a "phagosome". The changes in phagocytized Salmonella were observed by surrounding of the Salmonella with double layered structure of the plasma membrane, this alteration revealed degenerative changes in the bacterium cells while Yamamoto (6) observed triple layered structure of the plasma membrane which exhibited a degenerative changes of Salmonella enteritidis in MLNs of mice (6). The degeneration of the host cells was manifested by vacuolation of cytoplasmic displacement of organelles and swollen mitochondria. These changes in the host cells were occurred as the result of the effect of Salmonella endotoxin or metabolic substances from the Salmonella (6). Several membranes structure were surrounding some phagocytized Salmonella in the phagosome of macrophages, indicating that such Salmonella had been phagocytized by a host cell and then released subsequently to destroy the phagocyte, and phagocytized again by another cell (6). The presence of myeloid figure formation and the appearance of membranous structures showing unusual configuration as well as two whorls of concentric membrane which indicated the degeneration of the host cell. These changes were probably due to the autolytic effect of lysosomes which are promoted by an endotoxin. Salmonella typhimurium did not inhibit fusion of lysosomes with the phagocytic vacuoles in infected macrophages and caused no increase in cyclic adenosine 3' : 5' monophosphate (7). In murine Salmonellosis, the pathogen proliferates rapidly

in the organs of the reticuloendothelial system. It has been postulated that *Salmonella* multiply within macrophages of the host tissues and that acquired immunity, is primarily cell-mediated (8). Previous studies both in vitro (9,10,11) and in vivo (12,13) have shown that virulent *Salmonella typhimurium* is not killed but multiply slowly within macrophages.

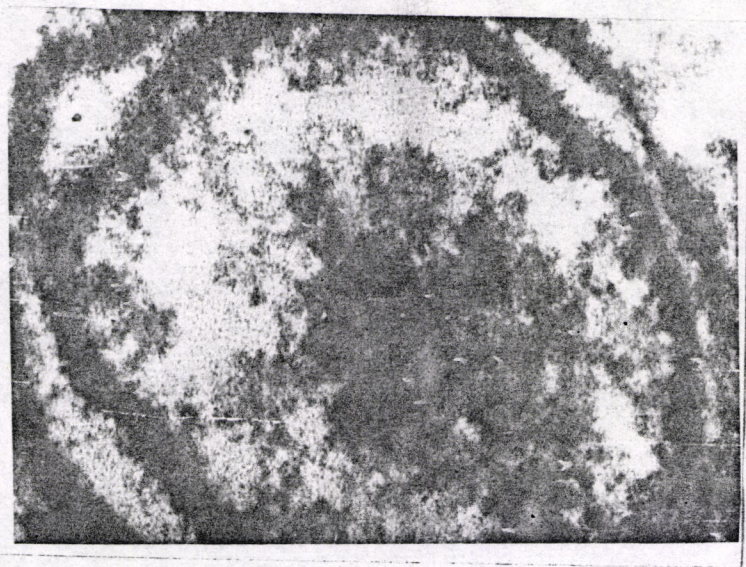


Fig. (1) : Double layered structure of the plasma membrane containing degenerating *Salmonella* in the macrophage at 24 hours (TEM). 50 X 50.

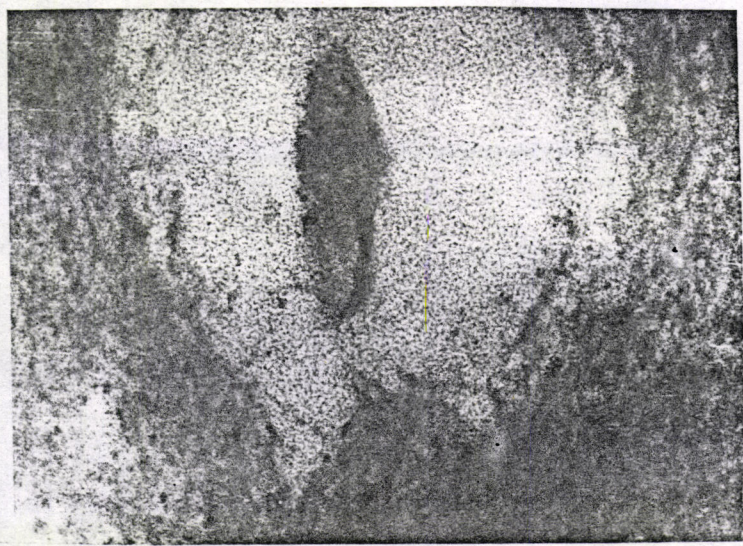


Fig. (2) : A higher magnification of phagocytized Salmonella in area of cytoplasmic vacuole inside the proliferative reticular cell (TEM). 50 X 100.

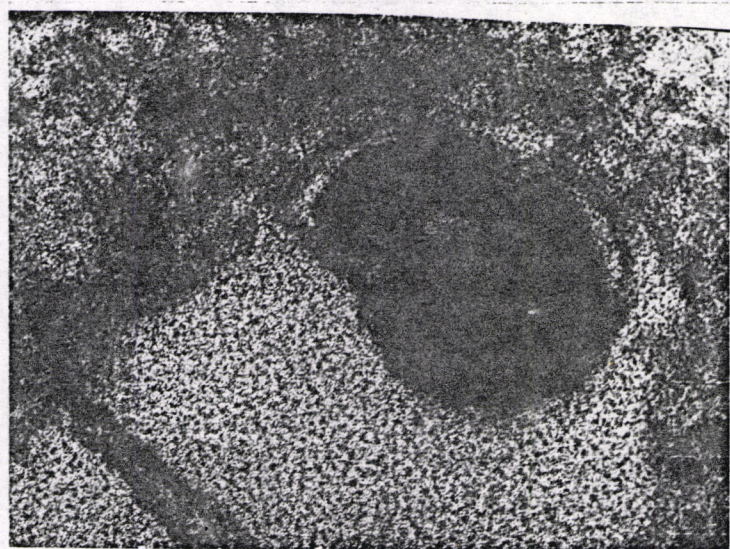


Fig. (3) : A higher magnification of myeloid figures in the cytoplasmic vacuole of degenerated cell (TEM). 20 X 200.



Fig. (4) : Two whorls of concentric membranes were observed inside mitochondria of the degenerated macrophage (TEM). 50 X 150.

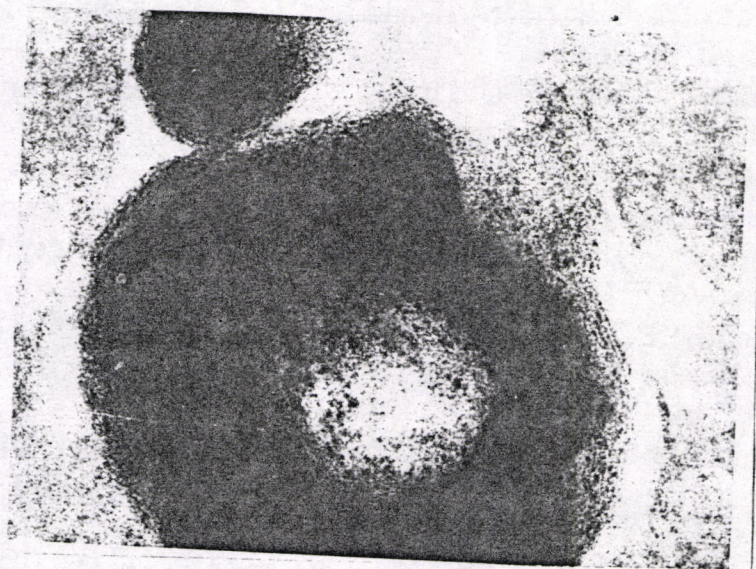


Fig. (5) : A higher magnification of two whorls of concentric membranes (TEM) 50 X 120.

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تغيرات التركيب الفوتي في العقد اللمفاوية في العجول المخمجة تجريبيا بجراثيم السالمونيلا تايفيموريم

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الخلاصة

صممت الدراسة لغرض معرفة جميع جوانب مرضية جراثيم
السالمونيلا تايفيموريم في العجول المخمجة تجريبيا. أجريت الدراسة على ستة
عشر عجلا من نوع الفريزيان ، سبق وأن تناولت اللبأ وبعمر ٣-٦ أسابيع.
قسمت العجول إلى مجموعتين ، أعطيت المجموعة الأولى جرعة من جراثيم
السالمونيلا تايفيموريم مقدارها (1.5×10^{11}) بينما عوملت المجموعة الثانية
كسيطرة. تميزت إصابة الغدد اللمفاوية بتمركز السالمونيلا داخل فراغات
هيولي خلاياها وأوضحت الدراسة أيضا العلاقة بين خلايا المضيف والبكتريا.