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HISTOPATHOLOGICAL EFFECTS OF EXPERIMENTAL INFECTION WITH *PSEUDOMONAS AERUGENOSA* IN THE LIVER AND LUNG OF MICE

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ABSTRACT

The histopathological effects of gram-negative bacteria *Pseudomonas aeruginosa* on mice liver and lung have been studied. Many histopathological alterations were found in the liver such as severe dilatations in the hepatic sinusoids and portal veins, degeneration and necrobiotic changes in the hepatocytes, proliferations in kupffer cells, some interspersed infiltration of inflammatory cells between the hepatocytes of the infected areas. Few Karyomegaly was observed in the nuclei of some hepatocytes. There were focal necrosis and vacuolar degenerations in most of the hepatocytes. Furthermore, oedema and dilatation in the bile duct and mononuclear leukocyte infiltration within the degenerative parenchyma associated with dilatation in the central veins were also observed. The hepatocytes of infected mice showed a reduced amount of total carbohydrates in comparison with control non-infected animals. The examination of the lung of infected mice showed interstitial inflammation, alveolar emphysema, giant alveolar formation, vascular invasion, hyperemia in the peribronchiolar, interalveolar and perialveolar blood vessels and capillaries when compared with untreated lung. Furthermore, acute pneumonia was detected in the infected lung. The present work suggested that most of the recorded pathological symptoms may be attributed to the toxic secretions of this pathogenic bacterium.

Keywords: Graw-megatiue bacteria, miceliner, micelung.

INTRODUCTION

Pseudomonas aeruginosa is a motile, rod-shaped , gram-negative, aerobic bacterium belonging to the family Pseudomonadaceae and it is a pathogen of humans. Genus *Pseudomonas* is found wherever organic matter is decomposing. Although, there are over 200 species of *Pseudomonas,* only three of them are known to be pathogenic for man (Todar, 2004 and Macia *et al.*, 2005). Almost all strains are motile by means of a single polar flagellum. These flagella yield heat-

labile antigens (H-antigen). The significance of antibody directed against these antigens, aside from its value in serologic classification is unknown (Iglewski, 2003). *P. aeruginosa* can grow well on most media such as blood agar plates or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor (Iglewski, 2003), in addition to its ability to grow at wide range of temperatures, 37- 42 °C (Jelsbak *et al.*, 2007. It is one of the top three causes of opportunistic human infections because it's highly resistant to antiseptics, disinfectants and antibiotics. It causes hepatototoxicity; chronic infection in the lung; bacteremic pneumonia; sepsis; urinary tract infections; gastrointestinal infections; dermatitis; bacteremia; soft tissue, bone and joint infections; and a variety of systemic infections, particularly in patients with severe burns that are associated with extremely high mortality (Arora *et al.*, 2005; Andy-Schaber *et al.*, 2007; Jelsbak *et al.*, 2007; Marr *et al.*, 2007).It produces virulent toxins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms. These toxins act as potent toxins that enter and kill host cells, near or at the site of colonization and to degradative enzymes causing a permanent disrupt of the cell membranes in various organs including liver and lung (Schumann, *et al.*, 1998; Hoffmann, *et al.*, 2005; Jelsbak *et al.*, 2007; Marr *et al.*, 2007). These virulence toxins include as polysaccharide capsule, anti-phagocytic; pili, adherence to the host cells; exotoxin "A", inhibit protein synthesis; elastase, destroying elastic fibers and blood vessels; and endotoxin LPS, toxic lipo-polysaccharide as well as phospholipase C and protease, break down lipids, lecithin and proteins facilitating tissue destruction (Iglewski, 2003 and Marr *et al.*, 2007) and increase mortality rate (Faur, *et al.*, 2003 & 2004).

The main purpose of the present work was to study the histopathological alterations induced in the liver and lung of mice experimentally infected with *P. aeruginosa* .

MATERIAL AND METHODS

Pseudomonas aeruginosa was kindly provided by Dr. M. Atta ,Faculty of Science, Cairo University. The bacterium was stored at -70 °C in brain-heart infusion broth supplemented with 10 % (v/v) glycerol and 5 % (w/v) skimmed milk until use (Yanagihara *et al.*, 2000). This bacterium was grown on standard laboratory culture media (Trypticase Soy Broth, TSB) at 37 °C in shaking water bath for 24 h. The microorganism was separated at its mid exponential phase. The microorganisms were suspended in saline, harvested by centrifugation (5000 rpm, 4 °C, 20 min), re-suspended in 100 ml sterile saline and adjusted to 1×10^6 cfu/ml

as estimated by tubidimetery. 5 ml from this suspension is centrifuged at 5000 rpm for 30 min. at 4 °C, the sediment bacteria were washed using sterile physiological saline, then were re-suspended in 5 ml to maintain the same concentration of the bacterial cells. This solution was used for injection.

Male, BALB/c, 7-week-old, 30-35 g. bodyweight, pathogen-free mice were purchased from Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. The animals were kept for a week in clean cages maintained under standard conventional conditions. Sixty BALB/c male mice were divided into 2 groups as follows, 12 mice, for group I and 48 mice for group II . Group I was injected IP with 0.1 ml sterile saline and kept out without infection as a control. Mice of group II were injected with 0.1 ml sterile saline containing active bacteria (Concentration 1×10^6 cfu/ml). The experiment was designed to dissect six animals selected randomly from both group I and II after 24 ,36 and 48 hours to detect the histopathological alterations. Mice of group II that died during the experimental periods were also used in the histopathological study.

The animals of group I and II were killed by cervical dislocation after the treatment. Their liver and lungs were excised, washed immediately by physiological saline then processed for histopathological and histochemical studies. Their liver were divided into two parts, one part fixed in Bouin's fixative for routine staining with Hematoxylin and Eosin. and the other one was fixed in Carnoy's fluid for staining using periodic acid Schiff's technique (PAS). The lungs were processed for H&E only. Fixed materials were washed, dehydrated and embedded in paraffin wax. Serial sections of 5μ thickness were cut then stained with hematoxylin and eosin for histopathological examination. Total carbohydrates were demonstrated in the liver using PAS technique (Hotchkiss ,1948). Selected slides were carefully examined and were photographed using Zeiss research microscope.

RESULTS

Mortality rate:

Table (1) showed the mortality rate in mice infected with *Pseudomonas aeruginosa.* The mortality rates were 46%, 58%, and 79% after 24, 36 and 48 hrs post-infection, respectively.

Table (1). Illustrated the mortality rate in the infected animals.

Hours post-infection	24	36	48
No. Mortality	22	28	38
Mortality rate	46%	58%	79%

Histopathological results:

Liver:

The liver of the control mice consists of hepatic lobules surrounded by portal areas having triads each of which includes bile ductule, one branch of both portal vein and hepatic artery. The blood flows from them through blood sinusoids into the central veins that are centrally situated in the hepatic lobules. The hepatocytes are radially arranged around the central veins. They have eosinophilic with finely granular cytoplasm. Most of the hepatocytes contain one nucleus and few of them have two nuclei (Fig.1).

After 24 hrs post-infection, examination of liver sections shows severe dilatation in the central and portal veins if comparison with that in the control liver. Degeneration and necrobiotic changes in the hepatocytes were observed (Figs. 2&3). At 36 hrs post-infection kupffer cells proliferation and inflammatory cells infiltration were noticed in between the degenerated hepatocytes in association with dilatation in the hepatic sinusoids. Karyomegaly was noticed in the nuclei of few hepatocytes (Figs. 4&5). Hyperemic central and portal veins were impacted by blood cells (Figs. 6&7). Moreover, there was focal area of necrobiotic changes in some hepatocytes in which the hepatocytes had deep basophilic pyknotic nuclei and eosinophilic cytoplasm (Fig.8). At 48 post-infection focal necrosis were observed in the hepatic tissue in diffused manner characterized by central zone of remnant basophilic materials of the necrosed nuclei associated with debris of eosinophilic cytoplasm as well as dilatation in the adjacent central veins (Figs.9&10). The hepatic parenchyma showed granular and vacuolar degenerations in most of the hepatocytes (Fig.11). Edema with few leukocytic infiltration and dilated bile duct were noticed in the portal areas (Fig.12). Also, degenerative and necrobiotic changes were detected in diffuse manner all over the hepatic parenchyma associated with dilatation in the central and portal veins (Figs.13&14).

A considerable amount of carbohydrates in the cytoplasm of liver cells of control animals was detected by PAS-technique. These carbohydrates give red or magenta colour with Schiff's reagent. It is noted that total carbohydrates is not uniformly distributed in the cytoplasm of some of the hepatocytes, but occurred concentrated at one pole of the cells (Fig.15). Examination of liver sections of infected mice at 36hrs post-infection shows necrotic areas surrounded by hepatocytes with reduced amount of carbohydrates (Fig.16). After 48 hrs post infection , the hepatocytes appeared with marked reduction of total carbohydrates (Fig. 17&18).

Lung:

The lung of the control group, showed no histopathological alterations, with normal histological structure of the air alveoli: bronchioles and peribronchiolar blood vessels (Fig.19).

Infected lung at 24 h post-infection shows sever hyperemia interalveolar and peribronchiolar blood vessels. Some of the emphysematous alveoli fused together to form giant alveolar formation. Interstitial inflammation, oedema and vascular invasion which observed near by the peribronchiolar blood vessels (Figs.20-22). At 36 post-infection, all pervious symptoms were recorded in addition to the interstitial inflammation increased in the peribronchiolar and interalveolar septa as well as severe degeneration were observed in most air alveoli. In the meanwhile degenerations were recorded in the walls of bronchi and peribronchiolar blood vessels (Figs23&24). Infected lung at 48 h post-infection. showed the presences of histopathological symptoms which explained before. although, a slight regeneration was detected. The histopathological alterations include oedema, interstitial infiltration, degenerated alveoli, emphysema and giant alveoli (Fig. 25). In addition, severe infected lung, at 48 h post-infection, shows acute pneumonia with chronic inflammation and the rest of the lung shows interstitial inflammation and congested blood capillaries in the walls of air alveoli (Figs. 26&27).

DISCUSSION

High mortality rates were recorded in mice infected with *Pseudomonas aeruginosa* in the present work. This findings strongly suggested that infected mice may be died under the influence of virulent factors released by *P. aeruginosa* which inhibit the immune response and increase tissue destruction. Similar high mortality rates were recorded by many authors (Davidson, *et. al.*, 1995; Heeckeren, *et. al.* 1997; Gosselin, *et. al.*, 1998; Sajjan, *et. al.*, 2001; Koehler, *et. al.*, 2003; Arora *et al.*, 2005; Haynes, *et. al.*, 2005; and Watanabe, *et. al.*,2007). Many reports demonstrated that most *P. aeruginosa* clinical strains had several virulent factors such as flagellin (Arora, *et al.*, 2005), pili (Saiman and Prince, 1993 and Tang *et al.*, 1995;Hertle, *et al.*, 2001), polysaccharide capsule (Iglewski, 2003), exotoxin-A (Cross *et al.*, 1980; Allured *et al.*, 1986; Pittet, *et al.*, 1996 ; Schümann *et al.*, 1998; Muhlebach, *et al.*, 1999 a; Hertle, *et al.*, 2001;), lipopolysaccharide "LPS" endotoxin (Iglewski, 2003), hemolysins, phospholipase C, leukocidin, protases elastases, (Iglewski, 2003 and Marr *et al.*, 2007;), cytotoxin Exo U (Faure, *et al*, 2003 and Hoffmann *et al.*, 2005), and. Exoenzyme S & Y (Yahr *et al.*, 1997&1998). These factors mediate adherence to the host

cells, antiphagocytic, inhibit protein synthesis (Schümann *et.al.,* 1998), killed polymorphonuclear leukocytes macrophages, lymphocytes, and other elements of immune system (Hertle, *et al.,* 2001), tissue damage (Jelsbak *et al.,* 2007), destroy elastic fibers and walls of blood vessels then finally lead to death (Pier, 2000; Faur, *et al.,* 2003 & 2004; Haynes *et al.,* 2005; Macià, *et al.,* 2005; McVay *et al.,* 2007; Marr *et al.,* 2007).

The present observations showed sever histopathological alterations in liver and lung of infected mice. From this point the present investigator suggested that toxins secreted by *P. aeruginosa* may inhibit or delay the immune response resulting in destruction and necrosis in the infected organs. Some investigators revealed that toxins of *P. aeruginosa* inhibit the immunological defenses and the resistance of host cells was significantly decreased (Cross, *et al.*, 1980;Schümann *et al.*,1998 and Yahr *et al.*, 1997&1998 ; Macià *et al.*, 2005).

Histopathological examination of liver showed kupffer cell proliferations, hemorrhage, degeneration and necrosis in the hepatic parenchyma as well as destruction in the epithelial wall of blood sinusoids, congestion of portal and central veins. These results are in agreement of many investigators who found injection of exotoxin-A extracted from *P.aeruginosa,* release of TNF and IFN into the circulation. inhibit protein synthesis especially in the liver and caused hepatocyte necrosis and apoptosis (Allured, *et.al.*, 1986; Misfeldt, *et.al.*, 1990 MacSween and Whaley, 1998; Schumann, *et.al.*, 1998; Muhlebach *et.al.*, 1999a). Schaber *et al.*, (2007) described two distinctive clinical features of *P. aeruginosa* bacteremia are invasion and necrosis of blood vessels .Watanabe *et al.*, (2007) revealed that systemic inflammation and inflammatory cytokine (IL-1ß, IL-6, and TNF-α) production increase significantly in the blood and livers of infected mice.

As regards to great depletion in polysaccharides in the infected liver, these findings explain the inhibiting action induced by cytotoxins. Schümann *et.al.,* (1998) documented that this pathogen caused sever hepatotoxicity accompanied by great depletion in polysaccharides and protein contents and finally result in severe or complete liver failure.

Regarding the effect of *Pseudomonas aeruginosa* on lung, many histopathological alterations were observed. Inflammatory cells infiltrate the interstitial tissue; blood vessels were dilated with vascular invasion to increase blood flow into the lung tissue. The toxic effects were established in the form of hemorrhage, emphysema, injure in the lining epithelia of the interalveolar blood vessel. These alterations were increased simultaneously with the increasing time of infection. Similar reports showed that this pathogen produced severe alveolar epithelial injury (Kudoh, *et.al.,* 1994; Sawa *et.al.,* 1999 and Wiener-Kronis, *et.al.,* 2001), chronic lung infection, airflow obstruction, (Sajjan, *et.al.,* 2001; Schaber, , *et.al.,*

2007 and Macià, *et.al.,* 2005), acute lung injury (Faur, *et.al.,* 2003 & 2004), pulmonary inflammation (George, *et.al.,* 1993; Khan, *et.al.,* 1995 and Muhlebach, *et.al.,* 1999 b), pneumonia (Dunn & Wunderink, 1995 Crouch-Brewer, *et.al.,* 1996 and Kurahashi, *et.al.,* 1999) and cystic fibrosis (Davisdon, *et.al.,* 1995; Noah, *et.al.,* 1997; Pier, 2000; Jelsbak, *et.al.,* 2007;) and death (Roy-Burman *et.al.,* 2001). Maciá *et al.*, (2005) found that *Pseudomonas aeruginosa* is the most relevant pathogen producing chronic lung infections with chronic underlying diseases such as cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease, finally it causes fast decline in the respiratory function. Imundo *et al.*, (1995) found that lung epithelia have specific receptor for this bacterium. It facilitates the adherence between this pathogen and the host cells. They anticipated that the epithelial injury increased in the lung parenchyma . It is suggested that the bacterium toxins prevent or inhibit the immune mechanisms to perform active role against this destructive bacterium. Published data in a good agreement with the present results. Toxins of this pathogen prevent the immune system of the infected mice to protect them and simultaneously lost its ability to kill *P.aeruginosa* (Faur *et.al.,* 2003; Heeckeren, *et.al.,* 2006). Some toxins such as Exo S, Exo T, Exo U & Exo Y caused epithelial injury, lung oedema, decrease in oxygenation and lung inflammation (Hoffmann *et al.*, 2005).

In conclusion, the present results proved that the pathogenecity of this bacterium result in tissue injury in the infected organs, killing or inhibit the immune cells and simultaneously delayed the immune response causing high mortality rates.

REFERENCES

Allured, V.S.; Collier, R.J.; Carroll, S.F. and McKay, D.B. (1986): Structure of Exotoxin A of Pseudomonas aeruginosa at 3.0- angstrom ngstrom Resolution. *National Academy of Sciences,* 83 (5): 1320-1324.

Andy-Schaber, J.; Triffo, J.W.; Suh, S.J.; Oliver, J.W.; Hastert, M.C.; Griswold, J.A.; Auer, M.; Hamood, A.N. and Rumbaugh, K.P. (2007): Pseudomonas aeruginosa Forms Biofilms in Acute Infection Independent of Cell-to-Cell Signaling. *Infection and Immunity,* 75 (8): 3715-3721.

Arora, S.K.; Neely, A.N.; Blair, B.; Lory, S. and Ramphal, R. (2005): Role of Motility and Flagellin Glycosylation in the Pathogenesis of Pseudomonas aeruginosa Burn Wound Infections. *Infect Immun.*, 73(7): 4395–4398.

Cross, A.S.; Sadoff, J.C.; Iglewski, B.H. and Sokol, P.A. (1980): Evidence for the role of toxin A in the pathogenesis of infections with Pseudomonas aeruginosa in humans. *J Infect Dis*

142:538- 546.

Crouch-Brewer, S.; Wunderink, R.G.; Jones, C.B. and Leeper, K.V. (1996): Ventilator-associated pneumonia due to Pseudomonas aeruginosa. *Chest*, 109:1019-1029.

Davidson, D.J.; Dorin, J.R.; McLachlan, G.; Ranaldi, V.; Lamb. D.; Doherty, C.; Govan, J. and Porteous, D.J. (1995): Lung disease in the cystic fibrosis mouse exposed to bacterial pathogens. *Nat Genet.*, 9:351-357.

Dunn, M. and Wunderink, R.G. (1995): Ventilator-associated pneumonia caused by Pseudomonas infection. (Review) *Clinics of Chest Medicine*. (16):95- 106.

Faure, K.; Fujimoto, J.; Shimabukuro, D.W.; Ajayi, T.; Shime, N.; Moriyama, K.; Spack, E.G.; Wiener-Kronish, J.P. and Sawa, T. (2003): Effects of monoclonal anti-PcrV antibody on Pseudomonas aeruginosa-induced acute lung injury in a rat model. *Journal of Immune Based Therapies and Vaccines*, 1:2 doi:10.1186/1476-8518-1-2. The electronic version: http://www.JIBTherapies.com/content/1/1/2

Faure, K.; Sawa, T.; Ajayi, T.; Fujimoto, J.; Moriyama, K.; Shime, N. and Wiener-Kronish, J. P. (2004): TLR4 signaling is essential for survival in acute lung injury induced by virulent Pseudomonas aeruginosa secreting type III secretory toxins. *Respir. Res.* 5:1.

Gosselin, D.; Stevenson, M.M.; Cowley, E.A.; Griesenbach, U.; Eidelman, D.H.; Boule, M.; Tam, M.F.; Kent, G.; Skamene, E. and Tsui, L.C. (1998): Impaired ability of Cftr knockout mice to control lung infection with Pseudomonas aeruginosa. *Am J Respir Crit Care Med.*, 157:1253- 1262.

George, S.E.; Kohan, M.J.; Gilmour, M.I.; Taylor, M.S.; Brooks, H.G.; Creason, J.P. and Claxton, L.D. (1993): Pulmonary clearance and inflammatory response in C3H/HeJ mice after intranasal exposure to Pseudomonas spp. *Appl Environ Microbiol.*, 59:3585-3591.

Haynes, A.; Ruda, F.; Oliver, J.; Hamood, A.N.; Griswold, J.A.; Park, P.W. and Rumbaugh, K.P. (2005): Syndecan 1 Shedding Contributes to Pseudomonas aeruginosa Sepsis. *Infection and Immunity*, 73 (12): 7914-7921.

Heeckeren, V.; Anna M.; Schluchter, M. D; Wei, X. and Pamela, D. B. (2006): Response to Acute Lung Infection With Mucoid Pseudomonas Aeruginosa in Cystic Fibrosis Mice. *Am J Respir Crit Care Med*,173: 288-296.

Heeckeren, A.; Walenga, R.; Konstan, M.W, Bonfield, T.; Davis, P.B. and Ferkol, T. (1997): Excessive inflammatory response of cystic fibrosis mice to bronchopulmonary infection with Pseudomonas aeruginosa. *J Clin Invest.*,100:2810-2815.

Hertle, R.; Mrsny, R. and David, J. and Fitzgerald, D.J. (2001): Dual-Function Vaccine for Pseudomonas aeruginosa: Characterization of Chimeric Exotoxin A-Pilin Protein. *Infect Immun.* 69 (11): 6962–6969.

Hoffmann, N.; Rasmussen, T.; Jensen, P; Stub, C.; Hentzer, M.; Molin, S.; Ciofu, O.; Givskov, M.; Krogh, H.; Johansen, H. and Høiby, N. (2005): Novel Mouse Model of Chronic Pseudomonas aeruginosa Lung Infection Mimicking Cystic Fibrosis, *Infection and Immunity,* 73(4): 2504-2514.

Hotchkiss, R.D. (1948): A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem. 16:131.

Iglewski B.H. (2003): Pseudomonas. In: Bacterial protein toxins, by: Drusilla L. Burns; Joseph T. Barbieri; Barbara H. Iglewski and Rino Rappuoli, 2003, Pub. Amer Socciety for Microbiology.

Imundo, L.; Barasch, J.; Prince, A. and Al-Awqati Q. (1995): Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface. *Proc Natl Acad Sci USA*, 92: 3019-3023.

Jelsbak, L.: Johansen, HK.; Frost, A-L; Thøgersen, R.; Thomsen, L.E.; Ciofu, O.; Yang, L.; Haagensen, J.A.J.; Høiby, N. and Molin S. (2007): Molecular Epidemiology and Dynamics of Pseudomonas aeruginosa Populations in Lungs of Cystic Fibrosis Patients. *Infection and Immunity*, 75 (5): 2214-2224.

Khan, T.Z.; Wagener, J.S.; Bost, T.; Martinez, J.; Accurso, F.J. and Riches, D.W. (1995): Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med.,* 151: 1075-1082, 1995

Koehler, D.R.; Sajjan, U.; Chow, Y.H.; Martin, B, Kent G, Tanswell AK, McKerlie C, Forstner, J.F. and Hu, J. (2003): Protection of Cftr knockout mice from acute lung infection by a helper-dependent adenoviral vector expressing Cftr in airway epithelia. *Proc Natl Acad Sci USA,*100: 15364-15369.

Kudoh, I.; Wiener-Kronish, J.P.; Hashimoto, S.; Pittet, J.F. and Frank, D. (1994): Exoproduct secretions of Pseudomonas aeruginosa strains influence severity of alveolar epithelial injury. *Am J Physiol*, 267:L551-L556.

Kurahashi, K.; Kajikawa, O.; Sawa, T.; Ohara, M.; Gropper, M.A.; Frank, D.W.; Martin, T.R. and Wiener-Kronish, J.P. (1999): Pathogenesis of septic shock in Pseudomonas aeruginosa pneumonia. *J Clin Invest*, 104:743-750.

Maciá, M.D.; Blanquer, D.; Togores, B.; Sauleda, J.; Pérez, J.L. and Oliver, A. (2005): Hypermutation Is a Key Factor in Development of Multiple-Antimicrobial Resistance in Pseudomonas aeruginosa Strains Causing Chronic Lung Infections. *Antimicrobial Agents and Chemotherapy*, 49 (8): 3382-3386.

Marr, A.K.; Overhage, J.; Bains, M. and Hancock, R.E.W. (2007): The Lon protease of Pseudomonas aeruginosa is induced by aminoglycosides and is involved in biofilm formation and motility. *Microbiology*, 153: 474-482.

MacSween, R.N.P. and Whaley, K. (1998): Microbial infections. In:Muir's text book of pathology, 13th ed. *Ed. By*: Roderick N.M. and Keith Whaley pp. 279-337. publishers: Arnold group: London, Sydney, Auckland

McVay, C.S.; Velásquez, M. and Fralick, J.A. (2007): Phage Therapy of Pseudomonas aeruginosa Infection in a Mouse Burn Wound Model. *Antimicrobial Agents and Chemotherapy*, 51 (6): 1934-1938.

Misfeldt, M.L.; Legaard, P.K.; Howell, S.E.; Fornella M.H. and LeGrand, R.D. (1990): Induction of interleukin-1 from murine peritoneal macrophages by Pseudomonas aeruginosa exotoxin A. *Infect Immun.*, 58(4): 978-982

Muhlebach, M.S.; Stewart, P.W.; Leigh, M.W. and Noah, T.L. (1999 a): Induction of interleukin-1 from murine peritoneal macrophages by Pseudomonas aeruginosa exotoxin A. *Infect, Immun.*, 58 (4): 978-982.

Muhlebach, M.S.; Stewart, P.W.; Leigh, M.W. and Noah, T.L. (1999 b): Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. *Am J Respir Crit Care Med*, 160: 186-191.

Noah, T.L.; Black, H.R.; Cheng, P.W.; Wood, R.E.: and Leigh, M.W. (1997): Nasal and bronchoalveolar lavage fluid cytokines in early cystic fibrosis. J Infect Dis 175: 638-647.

Pier, G.B. (2000): Role of the cystic fibrosis transmembrane conductance regulator in innate immunity to Pseudomonas aeruginosa infections. *Proc Natl Acad Sci USA*, 97: 8822-8828.

Pittet, J.F.; Hashimoto, S.; Pian, M.; McElroy, M.C.; Nitenberg, G. and Wiener-Kronish J.P. (1996): Exotoxin A stimulates fluid reabsorption from distal airspaces of lung in anesthetized rats. *Am J Physiol*, 270:L232-L241.

Roy-Burman, A.; Savel, R.H.; Racine, S.; Swanson, B.L.; Revadigar, N.S.; Fujimoto, J.; Sawa, T.; Frank, D.W. and Wiener-Kronish, J.P. (2001): Type III protein secretion is associated with death in lower respiratory and systemic Pseudomonas aeruginosa infection. *J Infect Dis*, 183:1767-1774.

Saiman, L. and Prince, A. (1993): Pseudomonas aeruginosa pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. J Clin Invest, 92: 1875-1880.

Sajjan, U.; Thanassoulis, G.; Cherapanov, V.; Lu, A.; Sjolin, C.; Steer, B.; Wu, Y.J.; Rotstein, O.D.; Kent, G. and McKerlie, C. (2001): Enhanced susceptibility to pulmonary infection with Burkholderia cepacia in Cftr(-/-) mice. *Infect Immun*, 69:5138-5150.

Sawa, T.; Yahr, T.L.; Ohara, M.; Kurahashi, K.; Gropper, M.A.; Wiener-Kronish, J.P. and Frank, D.W. (1999): Active and passive immunization with the Pseudomonas V antigen protects against type III intoxication and lung injury. *Nat Med,* 5:392-398

Schaber, J.A.; Triffo, W.J.; Suh, S.J.; Oliver, J.W.; Hastert, M.C.; Griswold, J.A.; Auer, M.; Hamood, A.N. and Rumbaugh, K. P. (2007): Pseudomonas aeruginosa Forms Biofilms in Acute Infection Independent of Cell-to-Cell Signaling. *Infection and Immunity*, 75 (8): 3715-3721.

Schümann, J; Angermüller, S.; Bang, R.; Lohoff, M. and Tiegs, G. (1998): Acute Hepatotoxicity of Pseudomonas aeruginosa Exotoxin A in Mice Depends on T Cells and TNF. *The Journal of Immunology*, 161: 5745-5754.

Tang, H.; Kays, M. and Prince, A. (1995): Role of Pseudomonas aeruginosa pili in acute pulmonary infection. *Infect Immun*, 63: 1278-1285.

Todar, K. (2004): Pseudomonas and related bacteria. Todar's Online Textbook of bacteriology. Written and edited by Kenneth Todar, University of Wisconsin-Madison Department of Bacteriology.

Wiener-Kronish, J.P.; Frank, D.W. and Sawa, T. (2001): Mechanisms of lung epithelial cell Injury by acute by Pseudomonas aeruginosa. In: Molecular biology of acute lung injury (*Edited by*: Clark RSG, Carcillo JA). Boston: Kluwer Academic Publishers pp. 149-161.

Yahr, T.L.; Mende-Mueller, L.M.; Friese, M.B. and Frank, D.W. (1997): Identification of type III secreted products of the Pseudomonas aeruginosa exoenzyme S regulon. *J Bacteriol*, 179:7165-7168.

Yahr, T.L.; Vallis, A.J.; Hancock, M.K.; Barbieri, J.T. and Frank, D.W. (1998): ExoY, an adenylate cyclase secreted by the Pseudomonas aeruginosa type III system. *Proc Natl Acad Sci USA, 95*:13899-13904.

Yanagihara, K.; Tomono, K.; Sawai, T.; Kuroki, M; Kaneko, Y.; Ohno, H.; Higashiyama, Y.; Miyazaki, Y.; Hirakata, Y.; Maesaki, S.; Kadota, J.; Tashiro, T. and Kohno, S. (2000): Combination theraby for chronic Pseudomonas aeruginosa respiratory infection associated with biofilm formation. *J. Antimicrobial Chemotherapy*, 46: 69-72.

LEGEND OF FIGURES

Fig. 1: Section of control liver shows portal areas containing bile ductules (BD), hepatic artery (HA) and portal vein (PV) which lined with simple squamous epithelium (SS). The central vein (CV) surrounded by hepatic cords (HC) of normal hepatocytes (H) and blood sinusoids (S) are observed between them. X 200.

Fig. 2: Section of liver of a mouse 24hrs post-infection showing dilated bile duct (BD) , congested central vein (CV), necrotic hepatocytes (NH), severely dilated portal vein (PV) and dilated blood sinusoids (S),. H&E, X 125.

Fig. 3: Section in liver of an infected mouse illustrating dilated central vein (CV), degenerated hepatocytes (DH), Erythrocytes (E), kupffer cell proliferation (K), necrotic areas (Ne), dilated sinusoids (S) and cytoplasmic vacuolation (V). X 250.

Figs. 4&5: liver of infected mice died at 36 hrs post-infection. These micrographs show two necrotic areas which had degenerated hepatocytes (DH), dilated blood sinusoids (DS), kupffer cell proliferation (K), and karyomegaly (Ka), leukocytic infiltration (Le) and necrotic hepatocytes (NH). Note that some hepatocytes have double nuclei (De). X 250.

Fig. 6: Liver section showing dilated bile ductule (BD); hyperemic central and portal veins (CV & PV), degenerated (DH) and necrotic hepatocytes (NH), cytoplasmic vacuolation (V). H&E, X 125.

Fig. 7: Section in liver of infected mouse showing degenerated and necrotic hepatocytes (DH & NH); kupffer cell proliferations (K); leukocytic infiltration (Le); dilated blood sinusoids (S) and cytoplasmic vacuolation (V). X 250.

Fig. 8: Liver section showing degenerated hepatocytes (DH), dilated sinusoids (DS), kupffer cell (K) proliferation ,focal necrosis (Ne), and cytoplasmic vacuolation (V). H&E, X 250.

Fig. 9: Section in liver of a mouse 48 hrs post-infection illustrating degenerated and necrotic hepatocytes (DH & NH); leukocytic infiltrations (Le); focal necrosis (Ne); necrotic tissue (NT); and dilated blood sinusoids(S). H&E, X 125.

Fig. 10: Magnified portion from figure 9 showing basophilic remnants of nuclei (BRN); degenerated and necrotic hepatocytes (DH & NH); dilated sinusoids (DS); eosinophilic remnants of cytoplasm (ERC); necrotic cytoplasm (NC). X 250.

Fig. 11: Liver section of infected mouse showing degenerated hepatocytes (DH); dilated sinusoids (DS); eosinophilic remnants of cytoplasm (ERC); granular and vacuolar degenerations (GD & VD). H&E, X 250.

Fig. 12: Section of liver showing severe dilatation in the bile duct (DBD) associated with huge number of inflammatory cells (IC) and noticeable oedema (Od). with few inflammatory cells (IC) infiltration and dilatation of the bile duct (BD) in the portal area. See also degenerated hepatocytes (DH) as well as dilated sinusoids (DS) and the lining epithelia were disappeared. H&E, X 125.

Fig. 13: Liver section showing bile ductules (BD); dilated central and portal veins (CV & PV); degenerative hepatocytes (DH) and dilated blood sinusoids (DS). H&E, X 125.

Fig. 14: Magnified micrograph from the previous figure showing bile duct (BD); degenerated hepatocytes (DH) and severe dilatation of the portal vein (PV). Note the degenerated epithelia of the bile duct (arrow heads) and portal vein (arrow). H&E, X 500.

Fig. 15: Section of control liver showing central and portal veins (CV&PV) surrounded with normal hepatocytes showed positive PAS reaction. Note that hepatocytes contain deep to moderate contents of glycogen materials. HG: high glycogen contents, MG: moderate glycogen contents. PAS technique, X 150.

Fig. 16: Section of liver 36 hrs post-infection showing several necrotic areas (NT) containing degenerated hepatocytes that were positively stained surrounded by hepatocytes with weak polysaccharide materials. PAS technique X 125.

Fig. 17: Light micrograph of infected liver 48 hrs post-infection showing weak reaction in hepatic lobules which formed of hepatocytes contains very low polysaccharide materials (LG). Note that many degenerated hepatocytes (DH) were interspersed between them. PAS technique, X 125.

Fig. 18: Magnified micrograph from the previous figure shows faintly stained hepatocytes containing low polysaccharide content and many degenerated hepatic (DH) cells. PAS technique, X 500.

Fig. 19: Light micrograph of control lung showing normal air alveoli (AA), bronchi (B) and peribronchiolar blood vessels (PB). H&E, X 125.

Fig. 20: Light micrograph of infected lung, 24 hrs post-infection showing emphysematous air alveoli (EM), giant alveoli (GA), hyperemic interalveolar blood vessels (IB), oedema (Od) surrounded some of the bronchi (B) and hemorrhaged (He) alveoli, interstitial inflammation (II). H&E, X 125.

Fig. 21: Light micrograph of infected lung showing emphysematous air alveoli (EM), giant alveoli (GA), interstitial inflammation (II), hyperemic

interalveolar (IB), perialveolar blood vessels (PA), vascular invasions (VI), oedema (Od) and hemorrhage (He). H&E, X 125.

Fig. 22: Light micrograph of infected lung of a mouse died at 24 hrs post-infection showing severe dilatation in a bronchus (B) and peribronchiolar blood vessel (PB) associated with oedema (Od) and degeneration in its walls. The rectangles defined the sites of degeneration. See also the presence of few small air alveoli (AA), hemorrhage (He), vascular invasions (VI), interstitial inflammation (II), emphysema (EM) and giant alveoli (GA). H&E, X 125.

Fig. 23: Light micrograph of infected lung, 36 hrs post-infection, shows few air alveoli (AA), severe degeneration in the wall of air alveoli (rectangles), in the wall of a bronchus (arrows), peribronchiolar blood vessel (arrow head). See also hemorrhage (He), vascular invasions (VI), oedema (Od), emphysema (EM) and giant alveoli (GA). H&E, X 125.

Fig. 24: Light micrograph of infected lung showing huge number of inflammatory cells (IC) in the peribronchiolar connective tissue associated with vascular invasion (VI) through which the inflammatory cells (IC) diffuse into the interstitial parenchyma forming interstitial inflammation (II). Note that few air alveoli (AA), severe degeneration in the wall of air alveoli (rectangles), in the wall of a bronchus (arrows), peribronchiolar blood vessel (arrow head). See also hemorrhage (He), oedema (Od), emphysema (EM) and giant alveoli (GA). H&E, X 125.

Fig. 25: Light micrograph of infected lung, 48 hrs post-infection showing few air alveoli (AA), severe degeneration in the wall of air alveoli (rectangles), in the wall of a bronchus (arrows), peribronchiolar blood vessel (arrow head). See also hemorrhage (He), vascular invasions (VI), oedema (Od), emphysema (EM) and giant alveoli (GA). H&E, X 125.

Fig. 26: Light micrograph of infected lung showing congested blood capillaries (CB), giant alveoli (GA) and acute pneumonia (P). H&E, X 60.

Fig. 27: Magnified micrograph from figure 25 shows emphysema (EM), giant alveoli, congested blood capillaries (CB), and interstitial pneumonia (IPn). H&E, X 100.

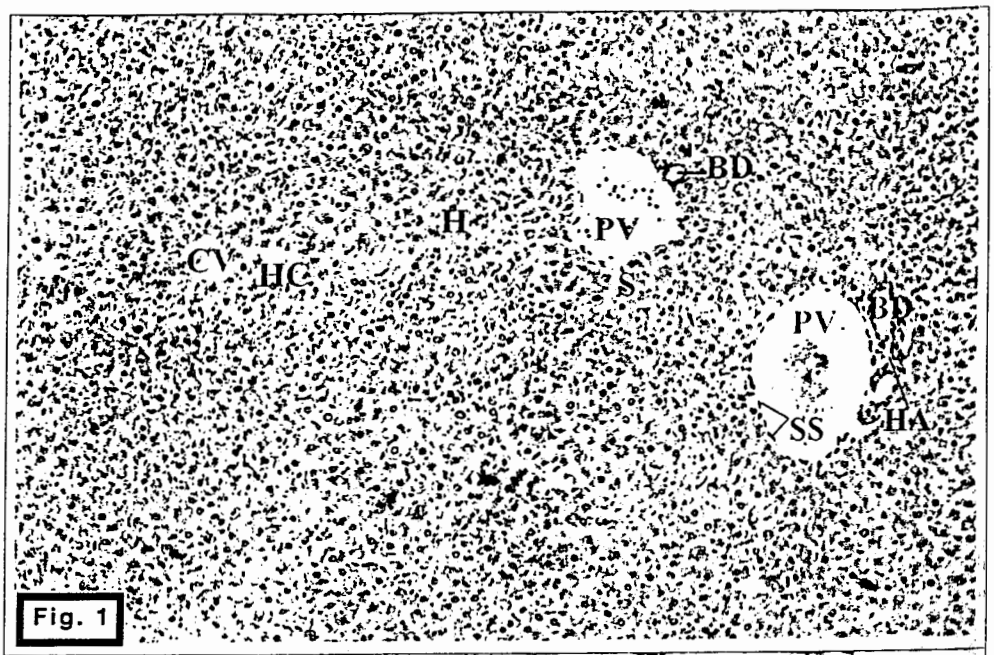


Fig. 1

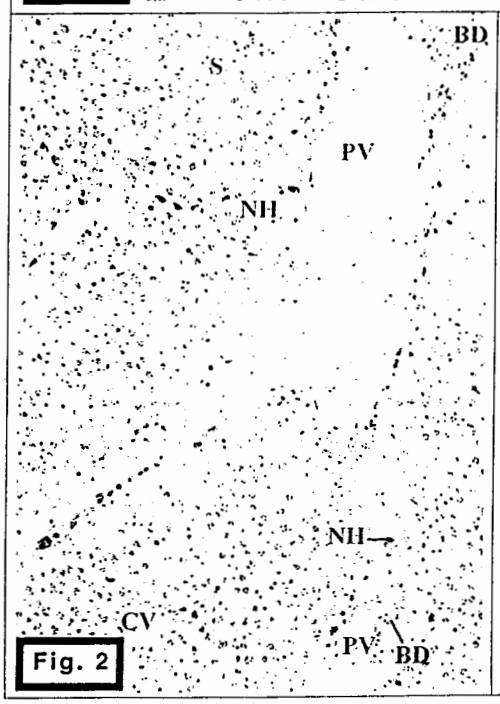


Fig. 2

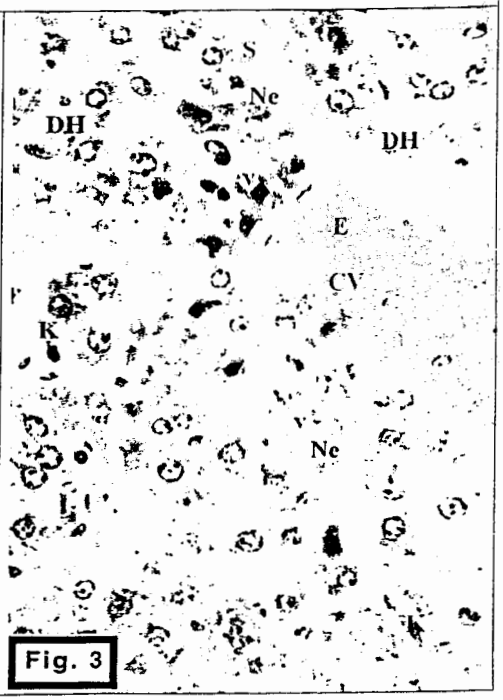


Fig. 3

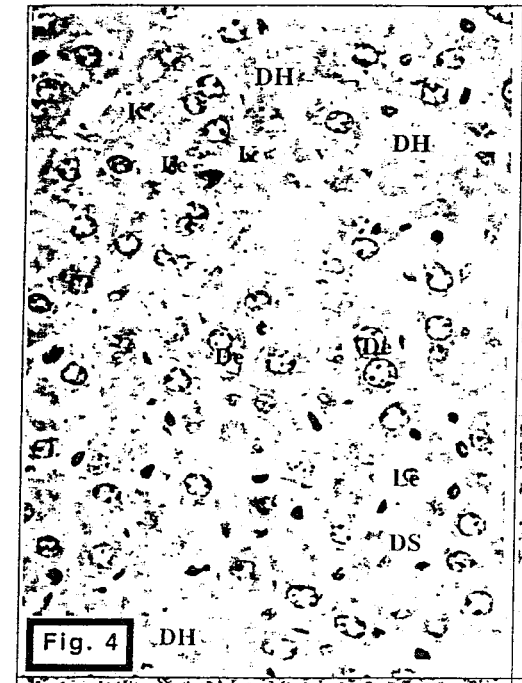


Fig. 4

Fig. 5

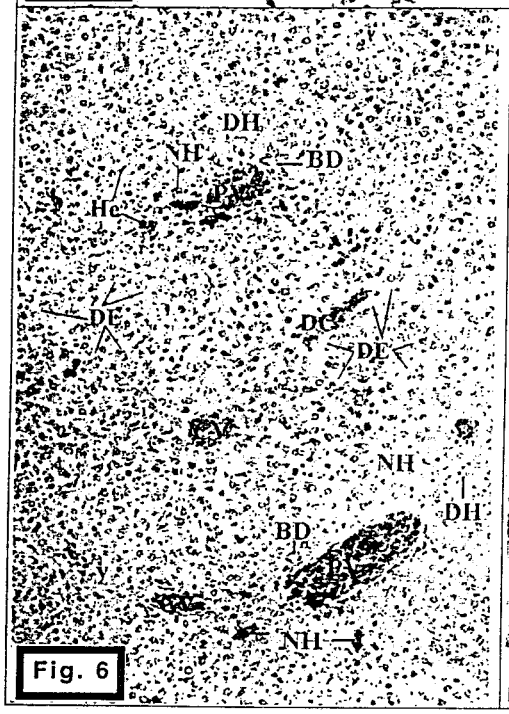


Fig. 6

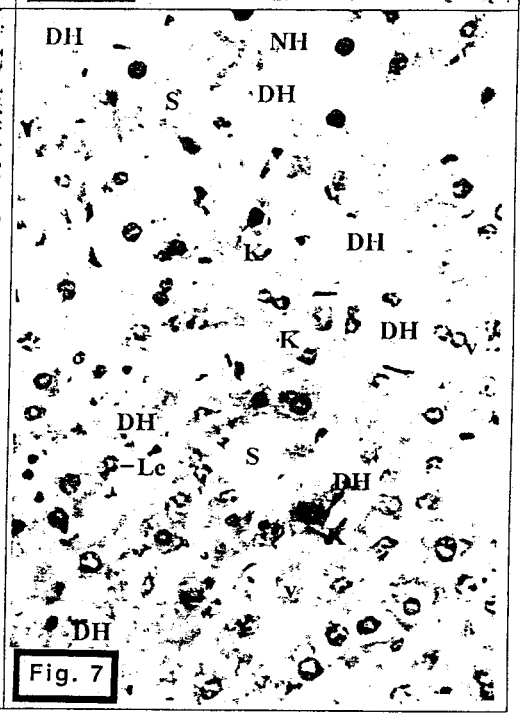
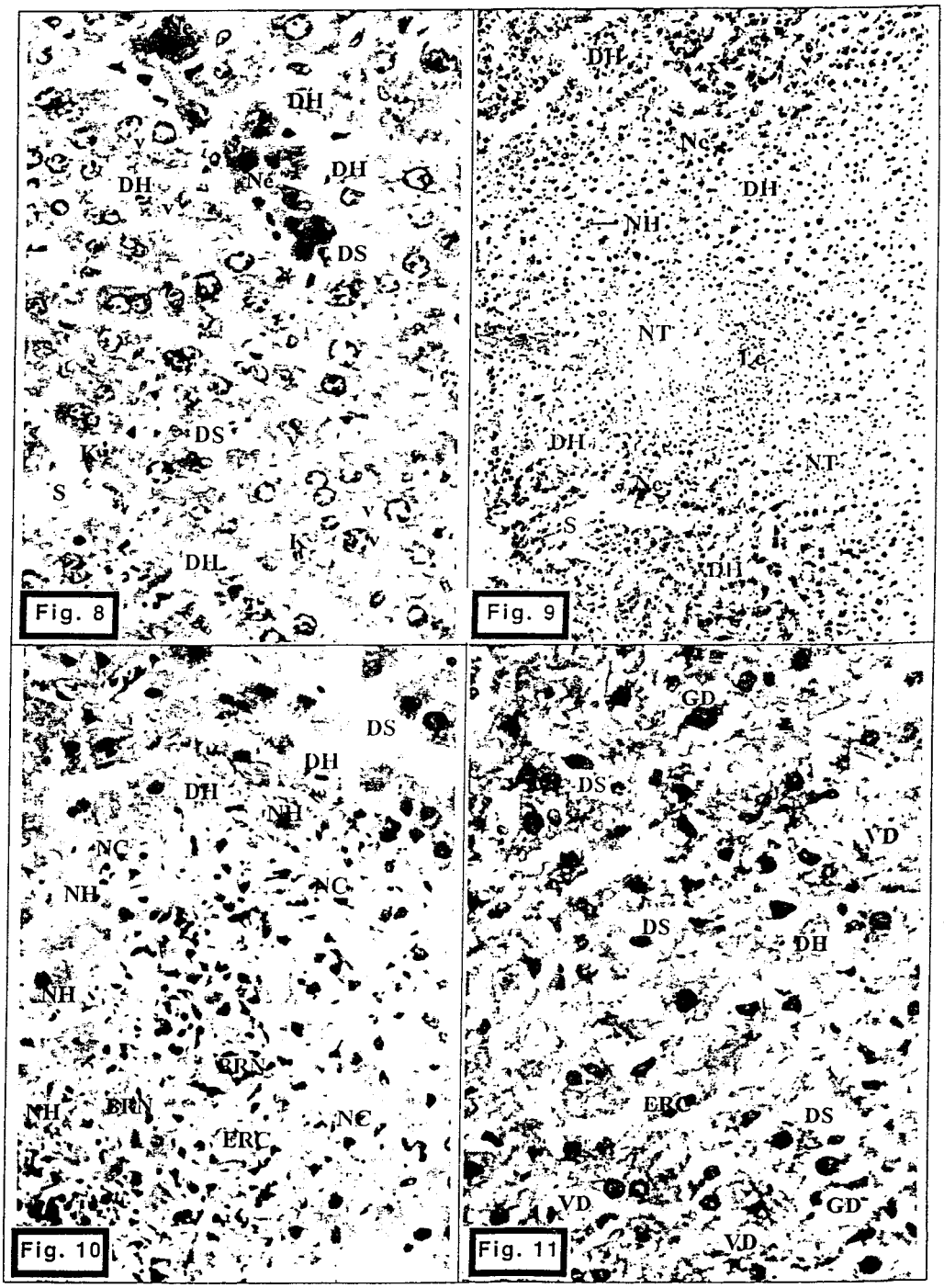


Fig. 7


DH
DH
Ne
DH
DS
DS
S
DH
Fig. 8
DH
Nc
DH
NH
NT
Tc
DH
NT
Nc
S
DH
Fig. 9
DS
DH
DH
NH
NC
NC
NH
NH
ERC
NH
ERC
ERC
NC
Fig. 10
GD
DS
DS
VD
DS
DH
ERC
DS
VD
GD
VD
Fig. 11


DH
NT
DS
DBD
Od
Od
IC
IC
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Fig. 12
DH
PV
CV
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PV
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PV
Fig. 13
DH
PV
BD
DH
Fig. 14

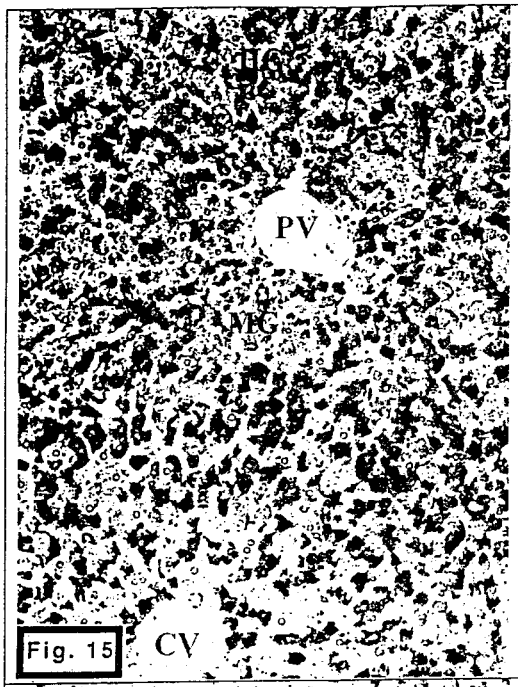


Fig. 15

Fig. 16

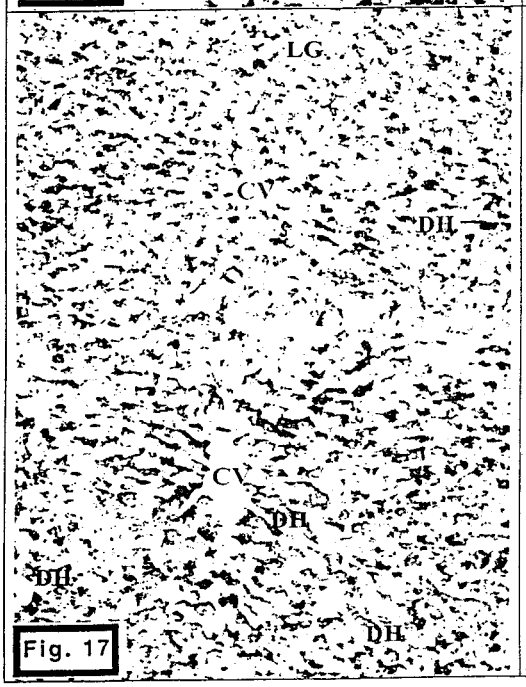
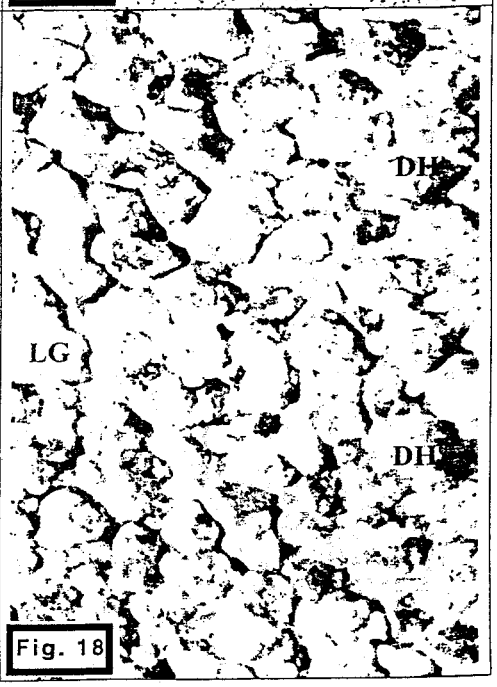


Fig. 17



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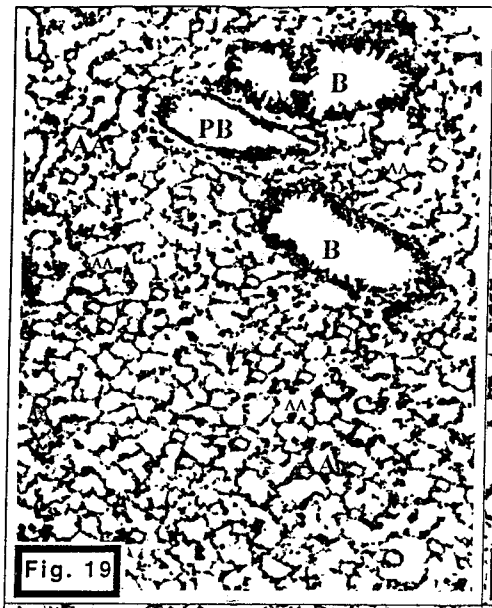
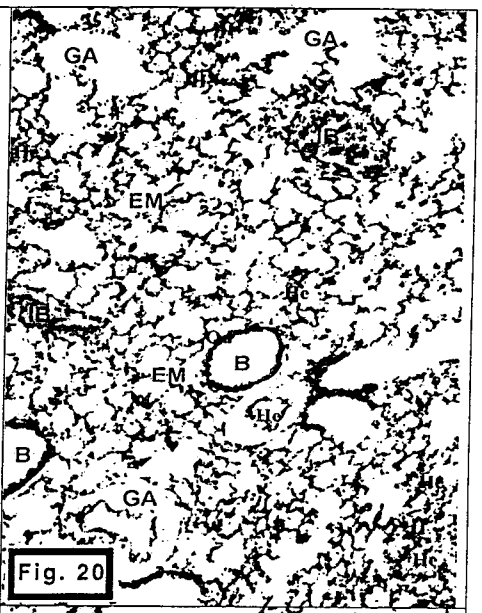
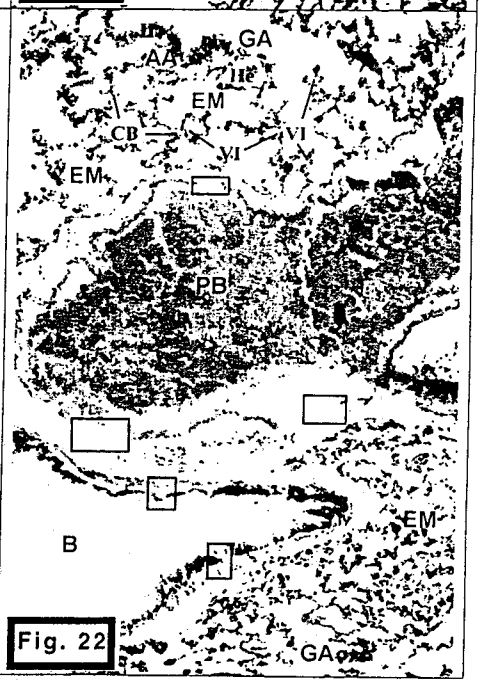

B
PB
B
Fig. 19


GA
GA
EM
B
EM
B
GA
Fig. 20


CB
AA
AA
IB
Od
VI
II
PA
CB
IIF
Od
VGA
VI
VI
He
EM
GA
Fig. 21


GA
AA
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CB
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VI
EM
PB
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EM
GA
Fig. 22

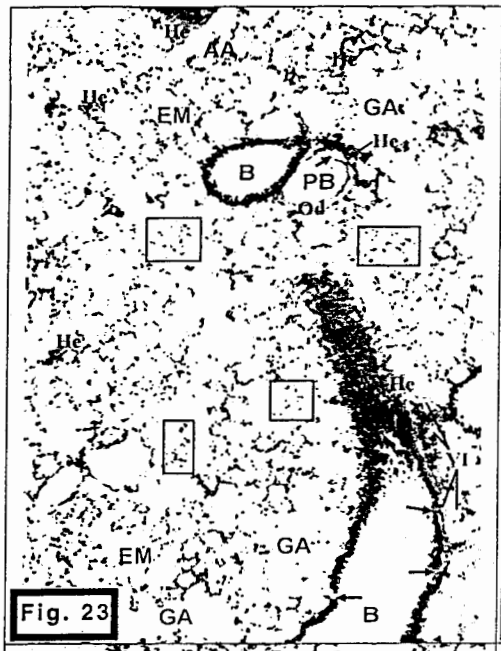

Fig. 23

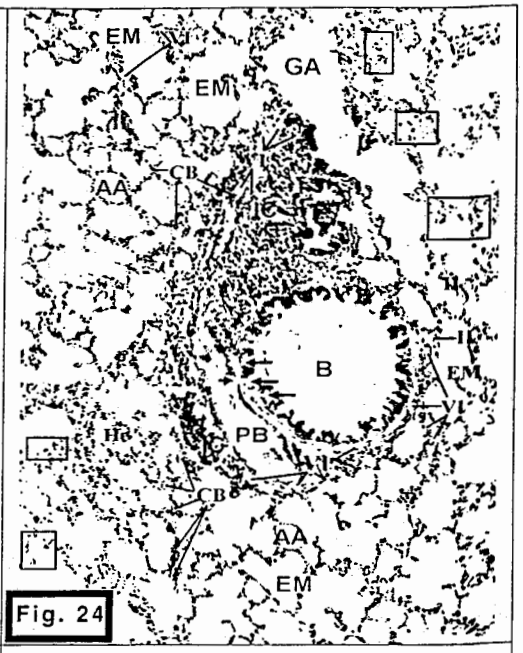

Fig. 24

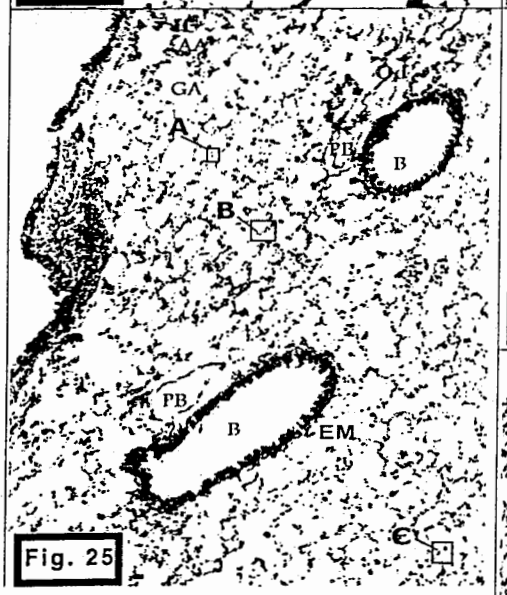

Fig. 25

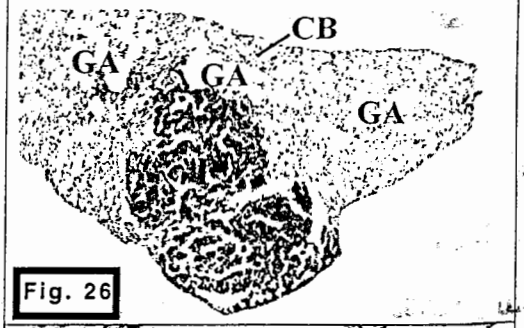

Fig. 26

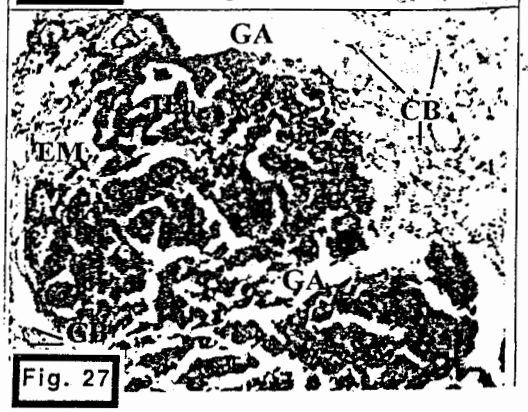

Fig. 27

OSAMA M. M. SARHAN

التأثير المرضي للبكتريا سالبة-الجرام، *سيدوموناس أوريوجينوزا* على كبد ورئة الفأر

أسامة محمد سرحان
كلية العلوم جامعة الفيوم

الملخص العربي

تم دراسة التأثير المرضي للبكتريا سالبة-الجرام، *سيدوموناس أوريوجينوزا* على كبد ورئة الفأر. بين الفحص الخارجي لكبد المجموعة المصابة، ظهور بقع باهتة أو شاحبة في الكبد. أظهر الفحص المجهري العديد من التغيرات المرضية، وتشمل اتساع حاد في الجيوب الدموية الكبدية، والأوردة البابية، انحلال وتنكس الخلايا الكبدية، انتشار خلايا كوفر، تسرب بعض الخلايا الالتهابية بين الخلايا الكبدية في المناطق المصابة. شوهد تضخم نووي في بعض أنوية بعض الخلايا الكبدية. ظهور تنكرز بؤري وانحلال فجوي في غالبية الخلايا الكبدية المصابة. وأخيراً، شوهد أيضاً إوديما، واتساع قناة الصفراء وارتشاح الخلايا البيضاء أحادية النواة بين برانشيما الكبد مصحوبة بتوسع في الأوردة المركزية. شوهد تفاعل PAS إيجابي في جميع الخلايا الكبدية المصابة في مناطق التنكرز. بينت نتائج فحص الرئة في المجموعة المصابة ظهور التهاب نسيجي، أنه يوجد انتفاخ حويصلي، تكوين حويصلات عملاقة، انتشار وعائي، وزيادة تدفق الدم سُجل في الأوعية والشعيرات الدموية حول شعيبية، وبين الحويصلية، وحول الحويصلية مقارنةً بالمجموعة الضابطة. علاوة على ذلك، ظهور التهاب رئوي حاد في الرئة المصابة.

وتخلص الدراسة أن جميع الأعراض المرضية السابقة ربما يعود سببها إلى الإفرازات السامة لهذه البكتريا الممرضة.