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METABOLIC SEQUELAE OF RESPIRATORY Q FEVER IN THE GUINEA PIG.(U)
AUG 77 M C POWANDA, S V MACHOTKA

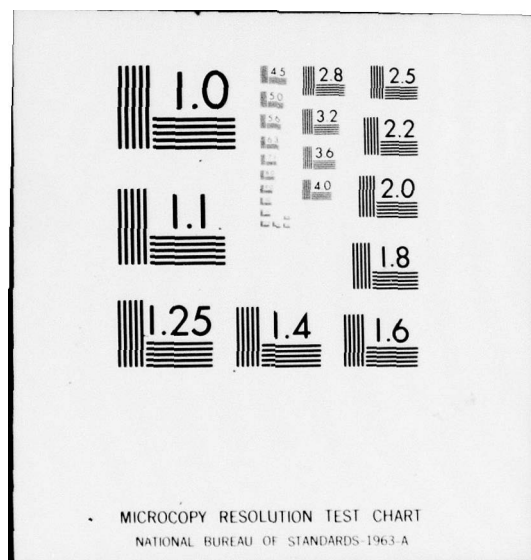
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6 Metabolic Sequelae of Respiratory Q Fever in
the Guinea Pig.

9 Interim rept.

10 M. C. POWANDA^{1*} S. V. MACHOTKA R. A. KISHIMOTO

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U. S. Army Medical Research Institute of Infectious Diseases

Frederick, Maryland 21701

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Running head: METABOLIC CHANGES WITH Q FEVER INFECTION

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

Guinea pigs infected with Coxiella burnetii administered in small-particle aerosols responded with a significant increase in plasma copper, seromucoid, and lysozyme on days 9 through 13. In contrast, plasma zinc decreased between days 7 through 13. These physiological changes paralleled increases in temperature, spleen and lung weights, and development of lesions.

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As part of a series of investigations into the nature and consequence of host-parasite interactions with Coxiella burnetii we studied certain aspects of host metabolism during infection. Previous studies showed biochemical alterations in guinea pigs following infection by the intraperitoneal route (8, 17). In the present study, guinea pigs were infected with C. burnetii administered in small-particle aerosols to simulate a natural respiratory exposure. The development of illness in guinea pigs is comparable to that observed in humans in respect to incubation period, severity and degree of mortality (11, 14, 25). Alterations in plasma trace metals, acute-phase globulins and enzymes have been used successfully to elucidate further the pathogenesis of certain infections (3, 4, 21). These metabolic alterations appear to be mediated by factors derived from leukocytes (9, 18), as a consequence of phagocytosis of tissue damaged during the course of the illness (21) or by the microorganisms themselves (M. C. Powanda and P. Z. Sobocinski, J. Cell. Biol. 67:343a, 1975). The data obtained from this study may aid in our understanding of the disease process in man during Q fever, as well as provide additional prognostic indicators for evaluating the efficacy of therapy and/or prophylaxis.

MATERIALS AND METHODS

Male Hartley strain guinea pigs, 300-400 g, were exposed to 10^4 median mouse intraperitoneal infectious doses (MIPID₅₀) of phase I Henzerling strain C. burnetii via small particle aerosol using a modified Henderson apparatus as previously described (11). Controls were exposed to sterile Earle's 199 medium. At designated times rectal temperatures were measured and the guinea pigs were then anaesthetized with halothane. The thoracic and abdominal cavities were opened, the inferior vena cava was transected and the blood accumulating within the pleural cavity was removed and placed in heparinized polypropylene tubes.

Plasma zinc and copper were analyzed by atomic absorption spectrophotometry (18), seromucoid by the procedure of Neuhaus et al. (13) and lysozyme by radial immunodiffusion (15). The presence of C. burnetii in tissue was determined after staining impression smears with a modified Giménez stain (10). Histopathological examination was conducted on tissue samples which had been fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

RESULTS

Preliminary study had revealed significant increases in both plasma seromucoid and copper concentration 8 days after aerosol exposure of the guinea pigs to C. burnetii (M. C. Powanda, G. T. Burger, G. H. Scott, and R. A. Kishimoto, Abstracts of the Annual Meeting--1977, Am. Soc. Microbiol. p. 24). The maximum response occurred on day 10 with seromucoid and copper concentrations of 3 to 4 times control values respectively, and the values returned to baseline by day 19. These transient perturbations in plasma copper and seromucoid concentrations paralleled the onset and persistence of fever.

A detailed study was therefore focused on the incubation and acute illness periods in an attempt to relate the observed metabolic alterations with specific aspects of the pathogenesis of the disease. Although both control and infected guinea pigs displayed some variability in body temperature, a significant increase in temperature of infected animals persisted from day 8 through day 13 (Fig. 1A). Spleen weight increased on day 7 and reached a maximum on day 11 (Fig. 1B) while lung weight began to increase on day 8 with maximal change on days 12 and 13 (Fig. 1C). The presence of rickettsiae in tissues is shown in Table 1. C. burnetii were detectable in almost all spleen samples from day 7 through day 13, while in lung there was a gradual accumulation beginning on day 8 with all animals becoming positive for C. burnetii on days 11 and 13. Rickettsiae were observed in some livers in the later stages of illness.

Tissues from guinea pigs necropsied on days 1, 2 and 3 postinfection were not significantly different from sham controls. Figure 2 presents changes in infected guinea pigs compared to normal lung (A). Five

days after exposure, guinea pigs developed a minimal to mild interstitial pneumonia with some exudation of macrophages and neutrophils into alveolar spaces (Fig. 2B). The interstitial pneumonia was more severe by 7 days, with significant exudation of neutrophils, macrophages and lymphocytes into alveolar spaces (Fig. 2C). By 9 days the lung lesions were severe with abundant exudation of fibrin, neutrophils, macrophages and lymphocytes (Fig. 2D). On day 11, the lesions were similar to those observed on day 9 except the inflammatory infiltrate was primarily composed of macrophages and lymphocytes (Fig. 2E). By day 13 consolidation was more extensive with infiltrate primarily composed of macrophages and lymphocytes (Fig. 2F). Early resolution became evident by day 15 and was nearly complete by day 29.

In addition to the pulmonary pathology, minimal to moderate splenic, hepatic and cardiac lesions were noted. Granulomatous splenitis and hepatitis became apparent by day 9 with persistence of the splenitis through day 15 and the hepatitis through day 29. Minimal lymphoreticular myocarditis was present by day 7 and continued through day 29.

Plasma seromucoid increased slightly on day 8, and was significantly elevated on day 9, it continued to increase through day 13 (Fig. 3A). Plasma copper was significantly increased on day 8, reached a peak on day 11, and declined thereafter (Fig. 3B). Plasma lysozyme was significantly increased on days 9 through 13 (Fig. 3C). Plasma zinc, in contrast to copper, displayed a transient decrease on day 3 and then began to decrease again on day 7, reached a nadir on day 11, and returned toward control values thereafter (Fig. 3D).

DISCUSSION

When guinea pigs are exposed to C. burnetii by aerosol there is no evidence of illness until 7 or 8 days after exposure. The onset of illness, as judged by a significant increase in body temperature, is also accompanied by an increase in the plasma concentration of seromucoid and copper and a decrease in plasma zinc. The occurrence of fever has typically been associated with the release of a substance, termed endogenous pyrogen, from various phagocytic cell types, including macrophages (1, 7). The seromucoid fraction of plasma consists of acute-phase globulins which, due to their carbohydrate content, are soluble in 0.6 M perchloric acid, but precipitable by the subsequent addition of phosphotungstic acid (13). An increase in seromucoid concentration normally occurs during inflammation (13, 21) and infection (2, 3, 22, 23) and can be produced by leukocyte derived factor(s) (LEM) (20). Plasma copper is, for the most part, a component of ceruloplasmin (5); increases in plasma copper during inflammation appear to reflect increases in this plasma protein (26). Plasma copper/ceruloplasmin can also be elevated by injection of leukocyte derived factors (19). A decrease in plasma zinc usually occurs during inflammation (21) and infection (3, 22, 23) and can also be precipitated by administration of LEM (20). Factors which can alter systemic host metabolism are not restricted to leukocytes; macrophages appear to release a substance or substances which can increase plasma haptoglobin concentration (16) and inhibit hepatic phosphoenolpyruvate carboxykinase (12) in mice. The increase in plasma lysozyme activity with a peak response on day 10 confirms the presence of inflammation (4) but, of course, does not specify the site of inflammation. The appearance of increased lysozyme

activity in the plasma has, in certain instances, prognostic value (3, 24). Macrophages, particularly activated macrophages, are capable of secreting lysozyme (6).

With the above in mind, it was conceivable that the alterations in plasma copper, zinc and seromucoid which were observed in guinea pigs beginning about 8 days postexposure to C. burnetii were the result of factors released from phagocytes, most likely macrophages, upon the ingestion of C. burnetii.

Although polymorphonuclear leukocytes can phagocytize C. burnetii (27) and thus give rise to the metabolic alterations observed during Q fever in the guinea pig, the fact that the metabolic sequelae appeared to await the development of interstitial pneumonia and to some degree to vary in intensity as a function of the incremental infiltration of macrophages into the lungs, implicated pulmonary macrophages as the predominant phagocytic cell type interacting with C. burnetii. Although the lung is the most likely site of macrophage-microorganism interaction, this does not preclude such from occurring in other tissues such as the spleen. The liver is, however, unlikely to contribute much in this respect, in that Q fever organisms can only be found in 50% or less of liver samples even late in illness yet all animals respond with marked metabolic alterations.

Proof that the interaction between phase I C. burnetii and macrophages gives rise to the metabolic alterations observed during Q fever in the guinea pigs will take time to acquire, but meanwhile these metabolic sequelae appear to be valuable prognostic indicators for evaluating the efficacy of therapy and/or prophylaxis.

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TABLE 1. Frequency of detection of rickettsiae in impression smears

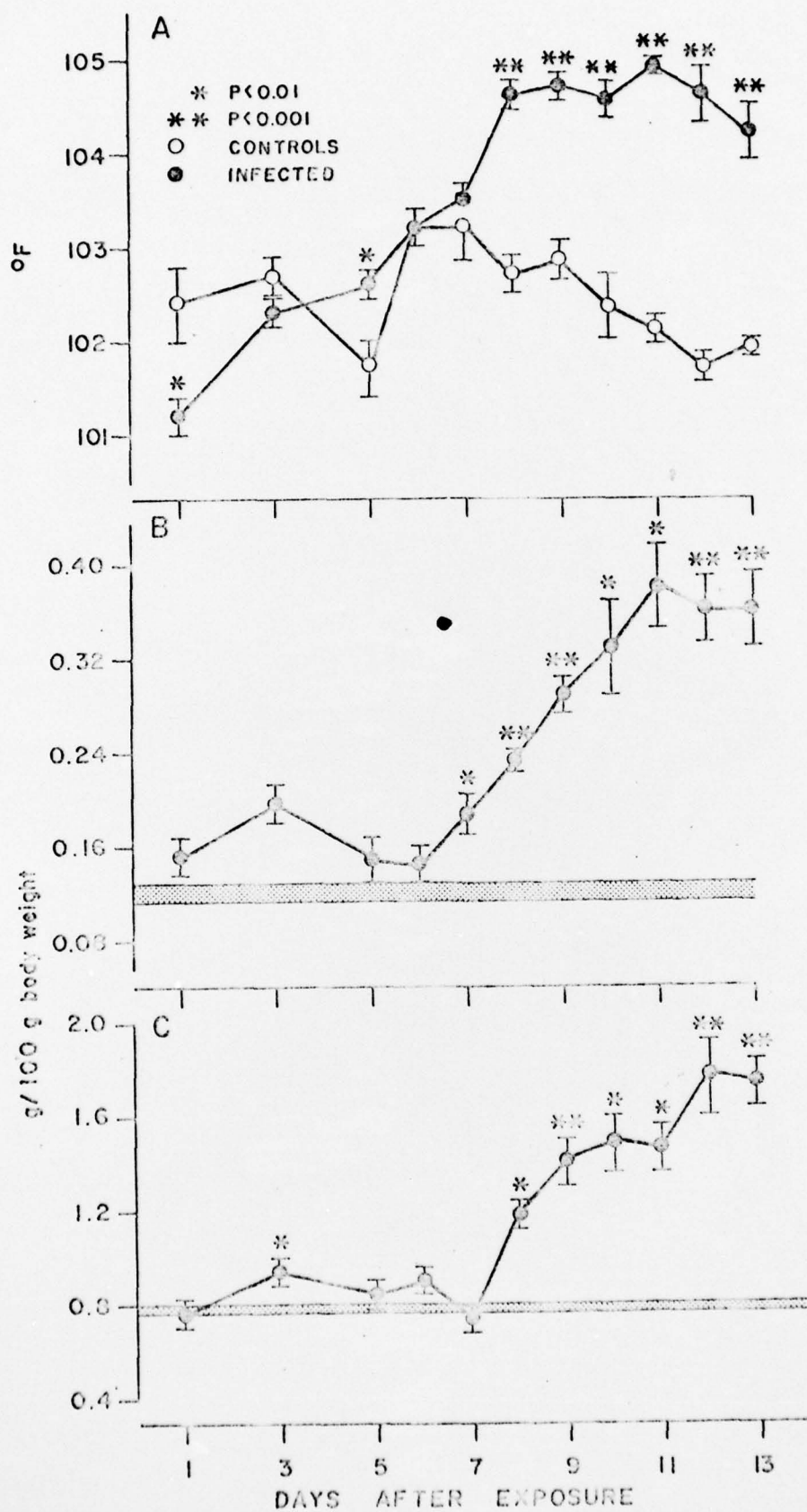
Tissue (n)	% Positive, by days									
	5	6	7	8	9	10	11	12	13	
Lung (7)	0	0	0	23	43	86	100	100	100	71
Liver (8)	0	0	0	0	0	0	38	50		38
Spleen (8)	0	0	100	83	83	83	100	100	100	100

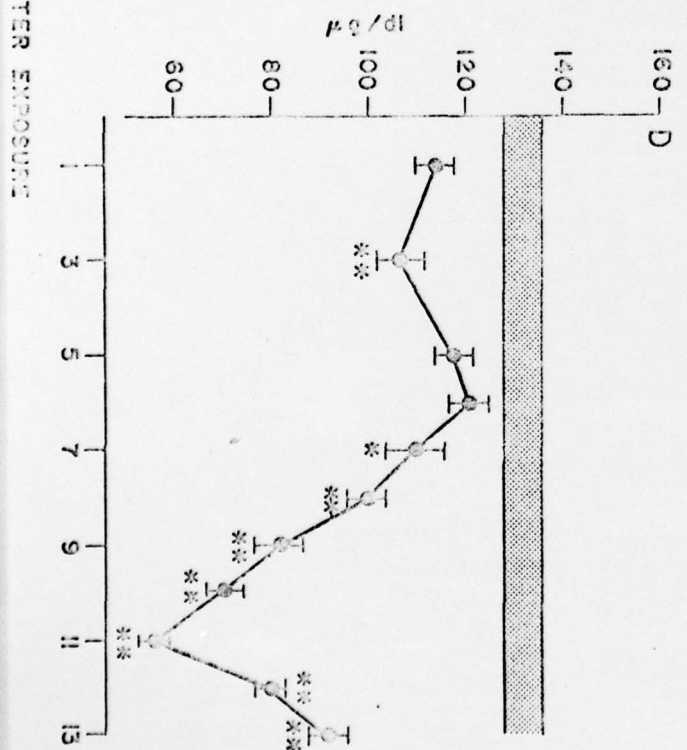
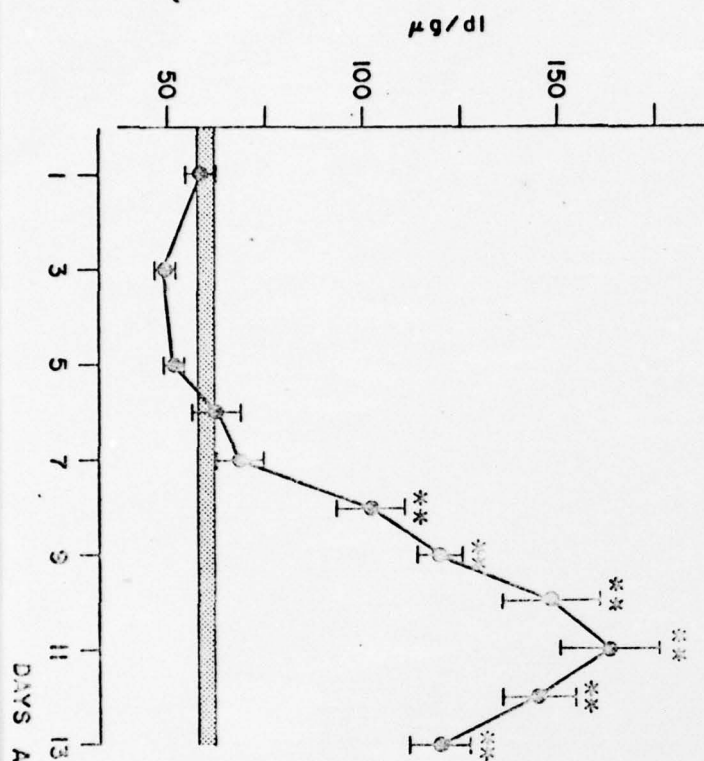
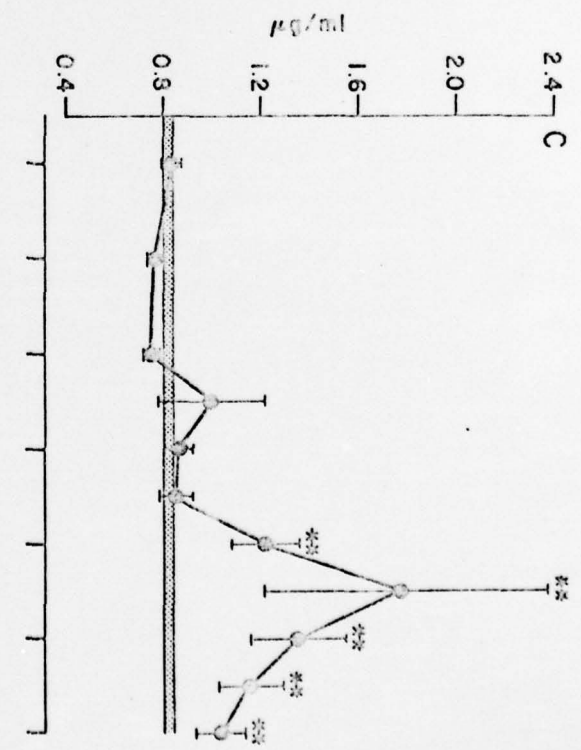
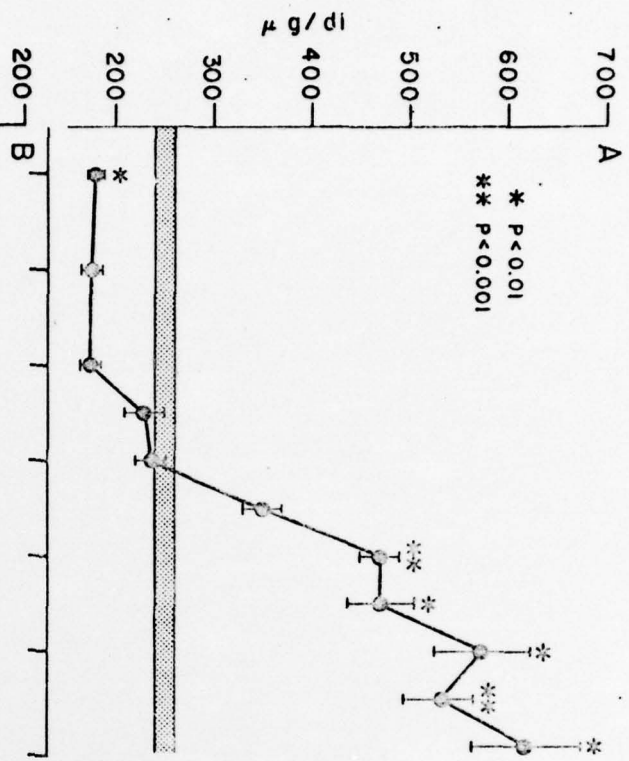
FIGURES

Fig. 1. Rectal temperature (A), spleen (B) and lung weight (C) following aerosol exposure of guinea pigs to C. burnetii are shown as daily means + SE values for the group. The variability in body temperature among control guinea pigs precluded these values from being summed together. Eight infected and 4 control animals were studied at each time point. The horizontal stipled bar in the lower graphs represents the mean + SE of values for 44 control guinea pigs. Analysis of variance was used to assess statistical significance.

Fig. 2. Photomicrographs of lung sections (X 240) from guinea pigs following aerosol exposure to C. burnetii hematoxylin-eosin stain. (A) Section of normal lung from sham control. (B) Interstitial thickening and mild exudate of macrophages and neutrophils into alveolar spaces 5 days postexposure. (C) Increased interstitial thickening with neutrophil, macrophage and lymphocyte exudation 7 days postexposure. (D) Pronounced exudation of neutrophils, macrophages and lymphocytes along with fibrin 9 days postexposure. (E) Accumulation of lymphocytes and macrophages in alveolar spaces and early consolidation 11 days postexposure. (F) Lymphocytes and macrophages filling alveolar spaces with more extensive consolidation 13 days postexposure.

Fig. 3. Plasma seromucoid (A), copper (B), lysozyme (C), and zinc (D) following exposure of guinea pigs to C. burnetii are shown as daily means + SE for the group. The stipled horizontal bars represent the mean + SE of the values from control guinea pigs. Analysis of variance was used to assess statistical significance.





DAYS AFTER EXPOSURE

