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Metabolic Sequelae of Respiratory Q Fever in the Guinea Pig[1] (40261)

MICHAEL C. POWANDA, S. V. MACHOTKA AND R. A. KISHIMOTO

U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701

As part of a series of investigations into the nature and consequences of host–parasite interactions with *Coxiella burnetii* (1, 2), we studied certain aspects of host metabolism during infection. Though previous studies showed biochemical alterations in guinea pigs following infection by the intraperitoneal route (3, 4), the relationship of these metabolic alterations to the pathogenesis of the disease was not clearly defined. In order to simulate a natural respiratory exposure, guinea pigs were infected with *C. burnetii* administered in small particle aerosols. The development of illness in guinea pigs was comparable to that observed in humans in respect to incubation period, severity and degree of mortality (2, 5, 6). We then measured plasma zinc, copper, seromucoid concentrations and lysozyme activity at various stages of the infection and attempted to relate changes in these parameters to organism concentration in selected tissues, as well as to the morphologic and histologic manifestations of the disease.

Materials and methods. Eighty-eight male Hartley strain guinea pigs, 300–400 g, were exposed to 10^4 median mouse intraperitoneal infectious doses ($MIPID_{50}$) of phase 1 Henzerling strain *C. burnetii* via small particle aerosol using a modified Henderson apparatus as previously described (2). Controls (44) were exposed to sterile Earle's 199 medium. All animals received water and Wayne laboratory guinea pig chow *ad libitum*. At designated times rectal temperatures were measured and the guinea pigs were then anesthetized 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 (7), seromucoid by the procedures of Neuhaus, et al. (8) and lysozyme by radial immunodiffusion (9). The presence of *C. burnetii* in tissue was determined after staining impression smears with a modified Gimenez stain (10) and by direct immunofluorescent antibody stain (11); two to three impression smears were taken of each tissue. 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

[1] 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.

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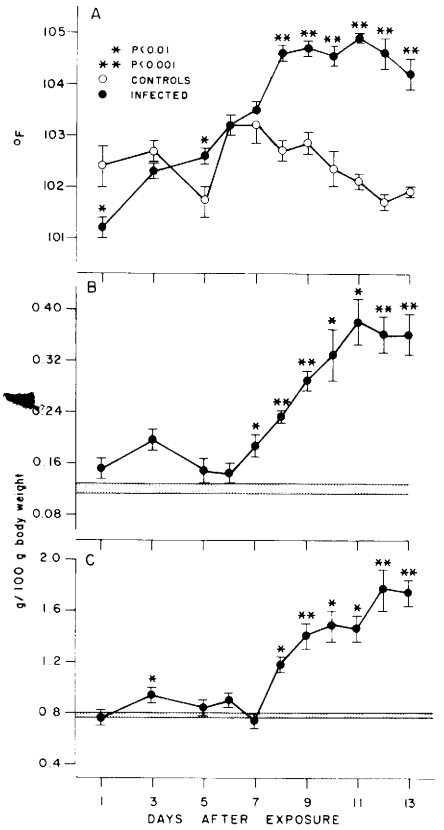

FIG. 1. Rectal temperature (A), spleen (B) and lung weight (C) following aerosol exposure of guinea pigs to *C. burnetii* are shown as daily means ± SE values for the group. The variability in body temperature among control guinea pigs precluded these values from being summed together. Eight infected and four 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.

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 I. *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 lyoszyme 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

TABLE 1. FREQUENCY OF DETECTION OF RICKETTSIAE IN IMPRESSION SMEARS.[a]

	Number positive, by days								
Tissue	5	6	7	8	9	10	11	12	13
Lung (7)	0	0	0	2	3	6	7	7	5
Liver (8)	0	0	0	0	0	0	3	4	3
Spleen (8)	0	0	8	7	7	7	8	8	8

[a] Eight animals were tested each day for the presence of rickettsia—one lung sample was taken for histologic examination; hence $n = 7$ in the case of the lung. Two to three impression smears were made of each tissue and these were examined by both Gimenez and direct fluorescent antibody stains.

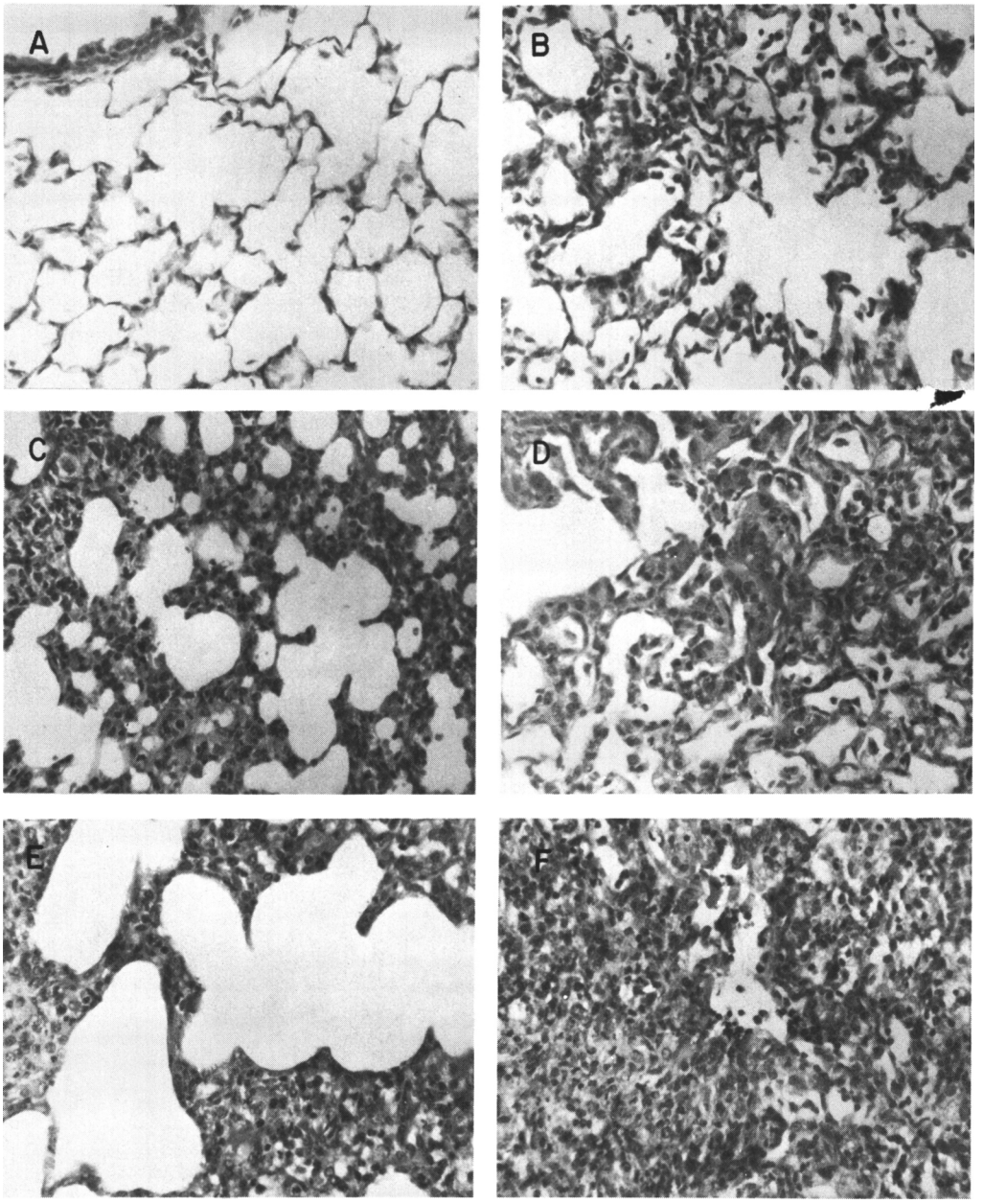

FIG. 2. Photomicrographs of lung sections (× 240) from guinea pigs following aerosol exposure to *C. burnetii* hematozylin-eosin stain. (A) Section of normal lung from sham control. (B) Interstitial thickening and mild exudate of macrophages and neutrophils into alveolar spaces 5 days postexposure. (C) 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 lymphphocytes and macrophages in alveolar spaces and early consolidation 11 days postexposure. (F) Lymphocytes and macrophages filling alveolar spaces with more extensive consolidation 13 days exposure.

on day 7, reached a nadir on day 11, and returned toward control values thereafter (Fig. 3D).

Discussion. Guinea pigs exposed to *C. burnetii* by aerosol appears to be a good model system to study the pathogenesis of respira-

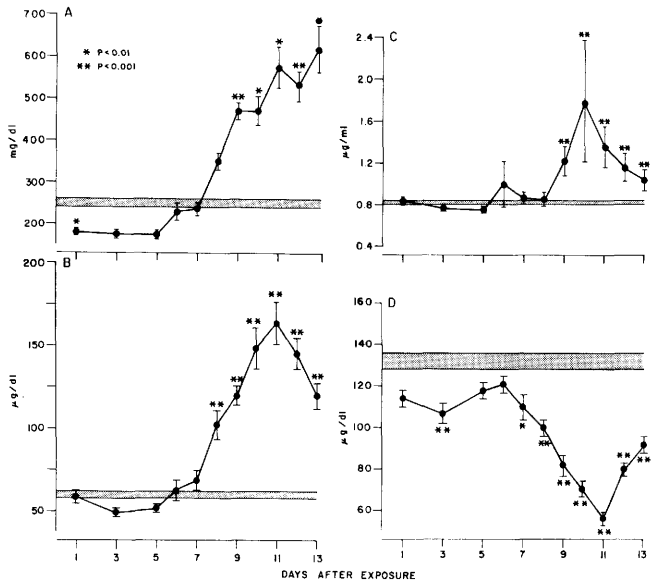

FIG. 3. Plasma seromucoid (A), copper (B), lysozyme (C), and zinc (D) following exposure of guinea pigs to *C. burnetii* are shown as daily means ± SE for the group. The stipled horizontal bars represent the mean ± SE of the values from control guinea pigs. Analysis of variance was used to assess statistical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

tory Q fever in that the development of illness in the guinea pigs is comparable to that observed in humans (2, 5, 6). Alterations in plasma zinc, copper and seromucoid occur during inflammation (8, 12) and infection (13–16). The zinc lost from plasma is largely that which is loosely bound to albumin (17) and appears to be stored in the liver (12, 14) in the form of Zn metallothionein (18). In acute illnesses decreases in plasma zinc appear to represent redistribution rather than deficiency (14). Plasma copper is, for the most part, a component of ceruloplasmin (19); increases in plasma copper during inflammation reflect increases in this plasma protein (20). Changes in seromucoid concentration are an effective index of acute phase globulin alterations in most circumstances. These alterations in plasma trace metals and proteins, as well as fever, appear to be mediated by a factor or factors derived from leukocytes, generally neutrophils (21, 22). However, factors which can alter systemic host metabolism are not restricted to neutrophils, macrophages appear to release a substance or substances which produce fever (23, 24), increases plasma haptoglobin concentration (25) and inhibit hepatic phosphoenol pyruvate carboxykinase (26).

With the above in mind, it was conceivable that the alterations in body temperatures, 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 macrophages upon the interaction with and/or ingestion of *C. burnetii.* Although polymorphonuclear leukocytes can phagocytize *C. burnetii* (27) and thus may 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 and perhaps to a lesser degree, the liver. The increase in plasma lysozyme activity with a peak response on day 10 may also be derived from macrophages, particularly activated macrophages (28) and confirms the presence of inflammation, (29) but of course does not specify the site of inflammation. Admittedly these studies only provide circumstantial evidence that the interaction be-

tween *C. burnetii* and macrophages gives rise to mediators which produce the metabolic alterations observed during Q fever in guinea pigs. *In vitro* studies will have to be conducted to ascertain whether and under what conditions macrophages may release mediators capable of altering host metabolism.

The present studies provide further evidence that the metabolic sequelae of infection display some degree of individuation. Rocky Mountain spotted fever, also a rickettsial disease, resulted in a significant increase in plasma copper in guinea pigs prior to fever and peak rickettsemia (15) while during Q fever the increase in plasma copper accompanied fever and the presence of rickettsiae in the lung. Moreover these studies may provide valuable prognostic indicators for evaluating the efficacy of therapy and/or prophylaxis in that the metabolic sequelae, if they are truly related to the pathogenesis of this disease, are likely to persist as long as the disease persists while clinical sign such as fever and rickettsemia can be obscured or abolished by antipyretic and antimicrobial therapy.

Summary. Guinea pigs infected with *Coxiella burnetii* administered in small particle aerosols provides a model for naturally acquired respiratory Q fever. Increases in plasma copper and seromucoid concentrations and in lysozyme activity along with a decrease in plasma zinc paralleled increases in body temperature, spleen and lung weights, and the development of lesions. Circumstantial evidence indicates that these metabolic alterations may be related to the interaction between pulmonary macrophages and the parasite. These metabolic sequelae thus may have prognostic value in that they appear related to the disease process and are less likely to be obscured than fever or rickettsemia.

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