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EXPERIMENTAL Q FEVER INFECTION IN CONGENITALLY ATHYMIC NUDE MIC--ETC(U)
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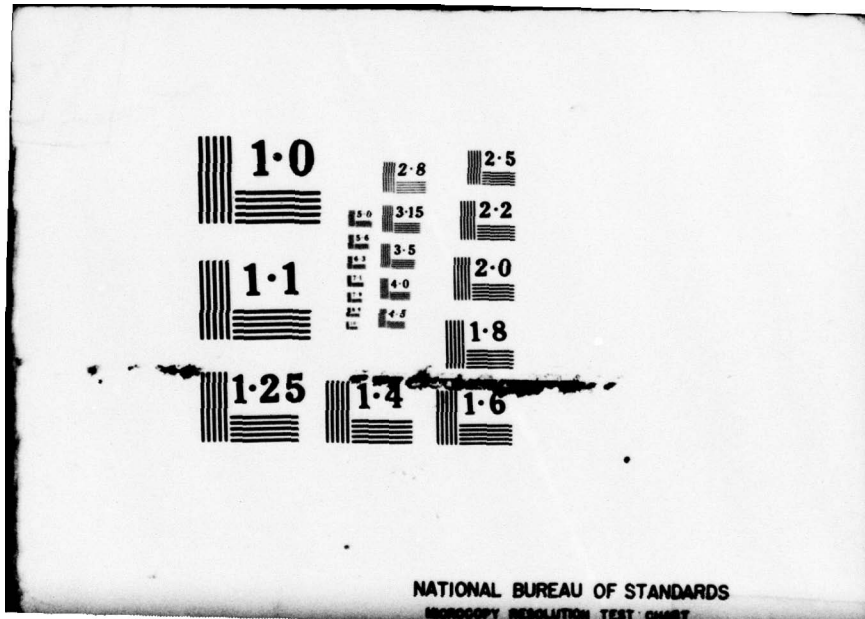
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Experimental Q Fever Infection in Congenitally Athymic Nude Mice

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ABSTRACT

Congenitally athymic nude (nu/nu) mice and their phenotypically normal (nu/+) euthymic littermates were exposed to Coxiella burnetii administered as small-aerosol particles. Following challenge, both strains of mice became infected, as characterized by rickettsemia, viable rickettsiae in the spleen, and serologic conversion. The major difference noted was that euthymic animals had cleared rickettsiae from peripheral circulation and the spleen within 14 days. In contrast, rickettsiae were detected and isolated from spleen and blood of athymic mice through 60 days.

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The host defense mechanisms which are involved in successful elimination of Coxiella burnetii from experimentally infected animals have not been fully elucidated. Previous in vivo studies suggest a role for immune serum. Abinanti and Marmion (1) showed that although immune serum is not known to have a direct rickettsicidal action on C. burnetii, passive transfer of serum from hyperimmune donors modified the infection in nonimmune animals. Although serum transfer can abrogate an infective challenge, the importance of specific antibody to an active Q fever infection is not known.

In addition to antibody, a role for cell-mediated immunity (CMI) in experimental Q fever had also been suggested. Peritoneal macrophages obtained from guinea pigs previously vaccinated with killed phase I C. burnetii were capable of phagocytizing and killing homologous rickettsiae in the absence of immune serum in vitro (11). Kazár et al. (10) demonstrated that spleen cells taken from mice immunized with live phase I C. burnetii transferred resistance.

While recent studies have shown thymus rudiment in athymic mouse (15) and high frequency of T-lineage lymphocytes in spleen (9), these animals have been shown to be devoid of functional T lymphocytes, which are required for the expression of CMI (16, 17, 19). Thus, the nude mouse provides a means for studying the role of CMI in host defense against Q fever.

This study was undertaken to assess further the relative importance of cellular and humoral immune systems in experimental Q fever infections. Congenitally athymic nude (nu/nu) mice and their phenotypically normal (nu/+) euthymic littermates were infected with C. burnetii to elucidate the role of humoral and thymus-dependent CMI resistance to Q fever infection.

MATERIALS AND METHODS

Preparation of rickettsial stock suspension. The third egg passage of the Henzerling strain of C. burnetii in phase I was grown in chicken embryo cells as previously described (11). The infectivity of the rickettsial suspension was estimated to be $10^{9.5}$ mouse median intraperitoneal infectious doses (MIPID₅₀).

Animals. Outbred Swiss (nu/nu), athymic mice and their phenotypically normal euthymic (nu/+) littermates, weighing 18-22 g, were obtained from the animal breeding facilities, Frederick Cancer Research Center. These animals had been bred and raised in a pathogen-free environment, and during the experimental period were housed in gas-tight, total-containment isolation facilities. All food (Purina 5010, Ralston Purina Co., St. Louis, MO), bedding and water acidified to pH 2.5 were sterilized prior to use.

Infection of mice. Mice were exposed to 10^4 MIPID₅₀ of the phase I Henzerling strain of C. burnetii presented in small-aerosol particles as previously described (13). Mice exposed to aerosols of sterile Earle's medium 199 served as controls.

Serologic assays. Serum antibody activity against phase I and II C. burnetii were evaluated at weekly intervals by the indirect immunofluorescent technique of Bozeman and Elisberg (2).

Rickettsemia. Blood from both strains of mice was collected at selected intervals and inoculated intraperitoneally into Swiss Webster mice. The animals were bled 21 days later and the presence of specific antibodies in serum was determined.

Spleen impression smears. The presence of C. burnetii in spleens of 3 to 5 mice was determined microscopically after staining two impression

smears with Giménez stain (6). Examination of two separate impression smears by direct immunofluorescent antibody technique (7) was employed as a confirmatory test. The amount of rickettsiae in spleens was estimated in ten different fields of view (X 1,000) and scored according to the method of Kazár et al. (10).

Viability of rickettsiae. The viability of rickettsiae in spleens was determined by inoculation of various dilutions of spleen homogenates into the yolk sac of 5-day-old chicken embryonated eggs. The surviving eggs were harvested after 10 days of incubation and yolk sac smears stained with Giménez stain and fluorescein isothiocyanate conjugated phase I and II C. burnetii antiserum.

RESULTS

Clinical signs and gross pathology. No clinical signs of illness (fever, weight loss, lethargy, coryza) or mortality were observed in any of the athymic or euthymic mice following exposure to C. burnetii. Gross lung lesions were observed from 7 through 14 days. These lesions appeared to be resolving by 21 days in both strains of mice. The spleen weight began to increase on day 5, and continued to increase until day 14 in both types of mice (Fig. 1). However, the spleen weight in euthymic mice declined to normal levels by day 21, whereas the spleen weights of athymic mice continued to increase; by day 30 the spleen weights were approximately 11 times heavier than those of control animals. On day 60 the weight of the spleen of athymic mice was approximately 5 times that of controls.

Isolation of rickettsiae from spleen and blood. The spleens from both strains of mice contained rickettsiae between 7 and 14 days as shown by the impression smears (Fig. 1). Rickettsiae were no longer detected in euthymic animals on day 21, but uncountable numbers of organisms per each field of view were observed in athymic mice for 60 days at which time the experiment was terminated. Titration of infected spleens in yolk sacs of embryonated eggs indicated that the spleens of athymic mice contained approximately 1×10^5 to 1×10^6 rickettsiae at 30 and 60 days, whereas no rickettsiae were recovered from euthymic animals at these times.

Rickettsiae were isolated from the blood of both athymic and euthymic mice on day 4 but organisms were no longer detected in euthymic mice by 21 days (Fig. 2). In contrast, rickettsemia persisted in athymic mice through day 60.

Antibody response. Antibody to phase I and II C. burnetii developed at the same rate in both strains of mice (Fig. 2). Antibody against phase II antigen was initially detected on day 14, and persisted at elevated titers through day 60. Antibody to phase I antigen was demonstrable by 21 days, and persisted at high levels. Uninfected control athymic and euthymic mice did not develop antibodies to either antigen.

DISCUSSION

These data demonstrate that both athymic and euthymic mice are infected following an aerosol exposure to 10^4 MIPID₅₀ of C. burnetii. The major difference noted was that euthymic animals were capable of clearing rickettsiae from peripheral blood and the spleen by 14 days, but athymic mice became chronically infected and did not eliminate the organisms. Studies by other investigators have also shown that infection of athymic nude mice with other intracellular microorganisms resulted in chronic infections (4,5,18).

While it is not known at this time what mechanism(s) underlie the observed phenomena it appears that the T lymphocytes may play a role in terminating Q fever infection in normal animals. Efforts are currently underway in our laboratory to determine the specific requirement for T lymphocytes by means of adoptive transfer studies. It is possible that soluble products of T cells (14) or macrophage inhibition factor (MIF) can activate macrophages and remove rickettsiae. Studies by Hinrichs and Jerrells (8) provide evidence that normal guinea pig peritoneal macrophages cultured in vitro with immune

lymphocytes from Q fever infected guinea pigs or with supernatant fluid rich in MIF inhibited the growth of ingested C. burnetii.

It appears that there are active nonspecific defense mechanisms in athymic mice, since none of the animals died following infection. Nonspecific defense mechanisms have also been reported in other infections. Cheers and Waller (3) noted that macrophages of nude mice are capable of being activated non-specifically during the course of Listeria monocytogenes infection.

Our results demonstrate that both athymic and euthymic mice are capable of producing antibodies against phase I and II C. burnetii. Since athymic mice were able to produce specific antibodies, it can be inferred that C. burnetii antigen elicits a T-cell independent humoral response. This antibody may act as an opsonin which then potentiates the destruction of rickettsiae by macrophages (11,12). However, antibody in concert with macrophages are not sufficient to terminate infection.

We believe that the athymic mouse may be a model for chronic and persistent Q fever studies.

ACKNOWLEDGMENTS

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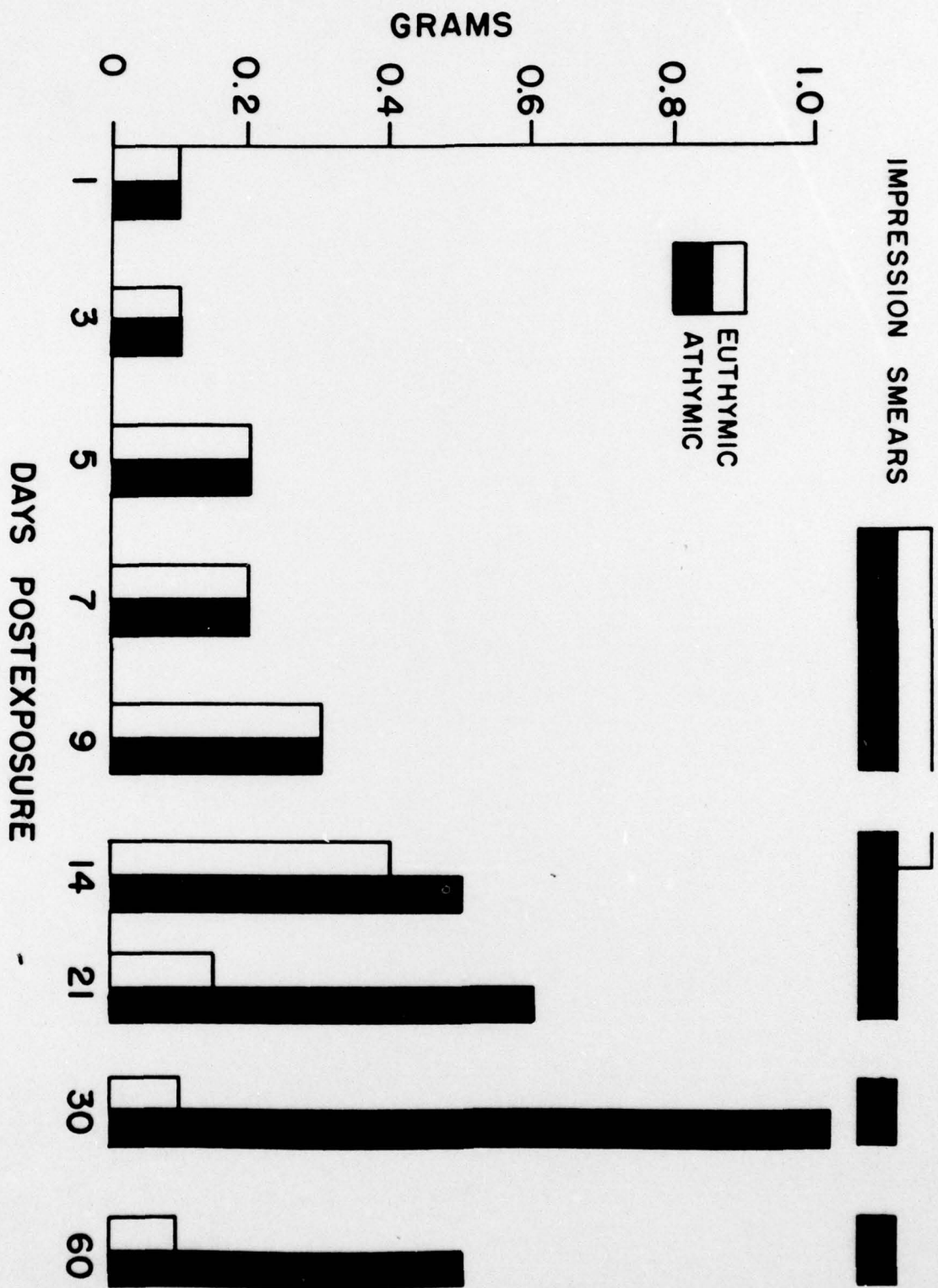
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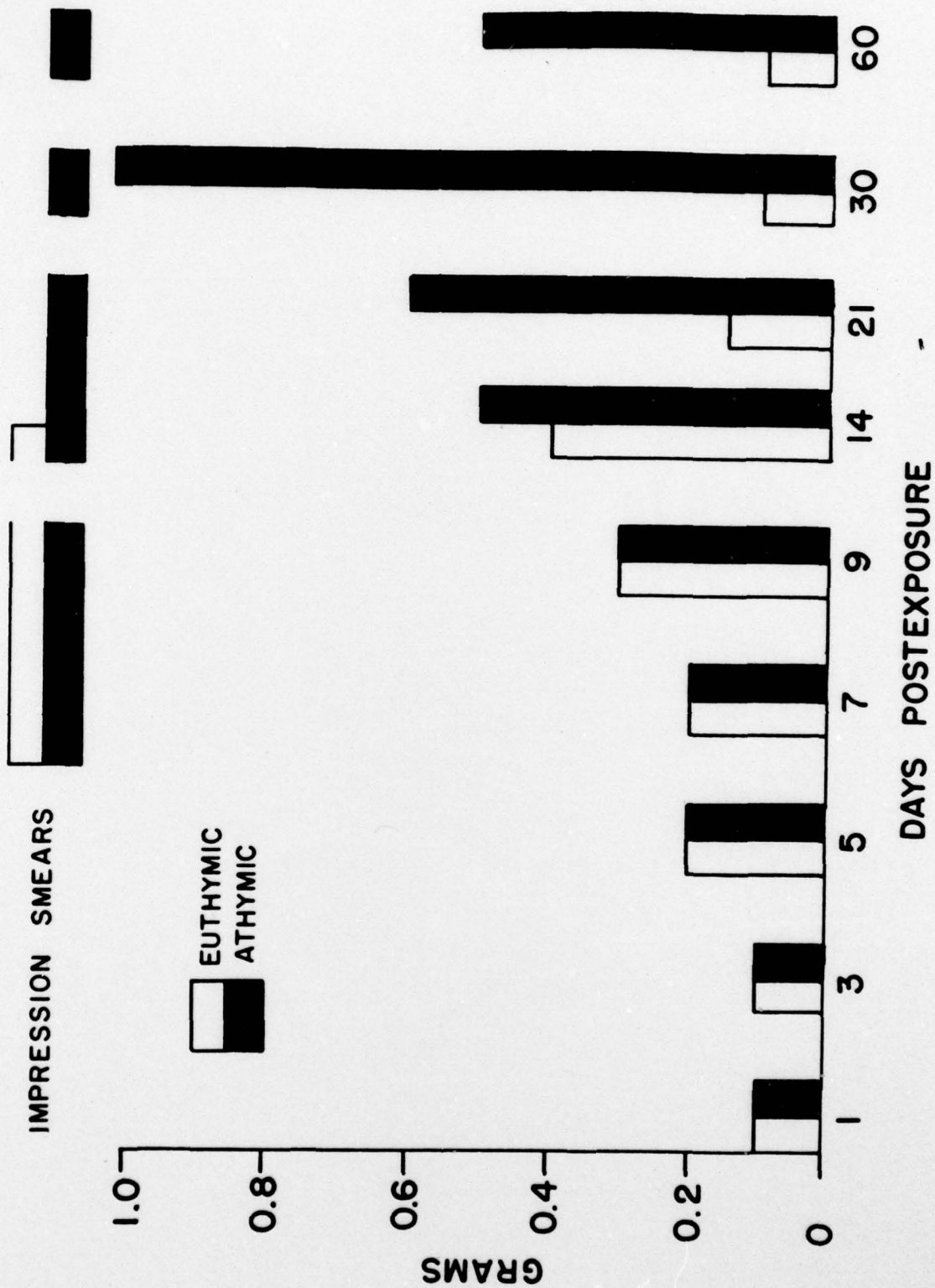
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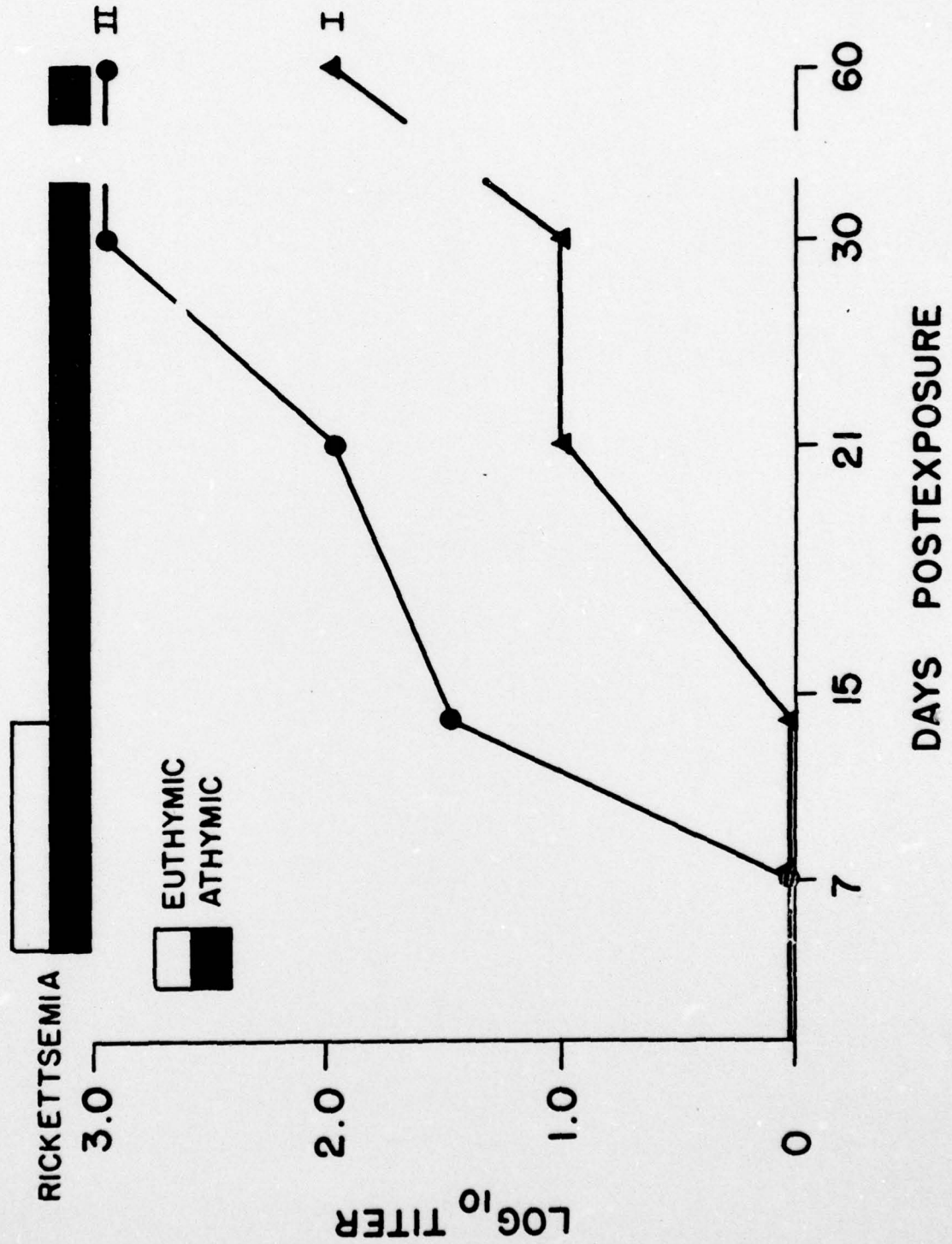
FIGURE LEGENDS

Fig. 1. Spleen weights and impression smears of athymic and euthymic mice following challenge with *C. burnetii*. Each point represents the mean of 3 to 5 animals.

Fig. 2. Onset and duration of rickettsemia and indirect fluorescent antibody titers of athymic and euthymic mice following challenge with *C. burnetii*. Each point represents the mean of 3 to 5 animals.







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