Swine were infected with Mycobacterium bovis to develop a model for pulmonary and disseminated tuberculosis in humans. Pigs were inoculated with various doses of M. bovis by intravenous (iv), intratracheal (int), or tonsillar routes. Animals were euthanized between 17 and 60 days after inoculation, and tissues were collected for culture and histopathologic examination. Lesions of disseminated tuberculosis were found in pigs given $10^4$ or $10^6$ cfu of M. bovis iv or int; localized pulmonary disease was found in pigs given $10^2$ or $10^3$ cfu of M. bovis int. Lesions ranged from well-organized tubercles with coagulative necrosis, epithelioid macrophages, and fibrosis to large expansive tubercles with liquefactive necrosis and extracellular growth of M. bovis. Tuberculous meningitis was observed in animals given M. bovis iv. Swine infected with M. bovis are a useful animal model for elucidating the mechanisms of pathogenesis and host defense to tuberculosis in humans.

Tuberculosis is the leading cause of death due to infectious disease in the world [1]. The incidence of clinical tuberculosis within a population is influenced by a number of factors. Clearly, the epidemic of human immunodeficiency virus infection has had a dramatic influence on the incidence of tuberculosis [1]. Other factors that suppress cell-mediated immune responses, acting alone or in combination with human immunodeficiency virus infection, to increase the incidence of clinical tuberculosis include intravenous drug abuse [2], alcoholism [3, 4], and malnutrition [5]. Study of the influence of drug abuse and alcoholism on the pathogenesis of tuberculosis is complicated by the lack of characterized animal models for studies involving a combination of these factors. Swine models of acute and chronic opiate abuse and of alcohol abuse have been well-characterized [6–8]. Therefore, establishment of a swine model of tuberculosis would provide a unique opportunity to study the influence of drug and alcohol abuse on the pathogenesis of tuberculosis.

Swine are natural hosts for mycobacterial infections, including those due to Mycobacterium bovis [9, 10]. The most common cause of swine tuberculosis is Mycobacterium avium, but infection with mammalian tubercle bacilli, including Mycobacterium tuberculosis, M. bovis, and Mycobacterium africanum, occur coincident with infections of cattle, wildlife, and human beings with these agents [9–12]. Lesions occurring in swine naturally infected with M. bovis and M. tuberculosis are indistinguishable [10]. Delayed-type hypersensitivity develops in pigs inoculated with M. bovis orally or intradermally [13, 14]. Swine and humans have similar patterns of resistance and susceptibility to virulent mycobacteria and develop lesions with a similar histologic character [15].

Swine make a convenient animal for experimental study because the animals are readily available and because their anatomy and physiology have proven to be remarkably similar to that of humans [16]. In addition, the immune system of swine has been well-characterized, and reagents are available for study of their immune responses [17]. Swine are being used increasingly as animal models for biomedical research applications in fields such as cardiology, physiology, behavior, pharmaceutics development, and organ transplantation. Furthermore, swine are large enough to allow repeated blood sample collection, tissue biopsy, and other surgical procedures with little harm to the animal.

Other animal models for the study of human tuberculosis include rabbits [18, 19], mice [20], and guinea pigs [21, 22]. These species are not natural hosts for infection with mammalian tubercle bacilli but can be infected experimentally. Rabbits and guinea pigs are more susceptible to infection and disease than are human beings; mice are more resistant [15].

Elegant pathogenesis and descriptive studies have been conducted in rabbits, and much of what is known about the development of the early lesions of tuberculosis can be ascribed to those studies. Lurie [23] and Dannenberg [24] described the lesions and pathogenesis of M. bovis infection in inbred strains of rabbits that were either susceptible or resistant to infection.
Although these inbred lines are no longer available for study, rabbits are used as a model of pulmonary tuberculosis and in studies of the pathogenesis of cavitary disease [19, 24, 25].

Guinea pigs have been used extensively in mycobacterial research because of their exquisite susceptibility to infection with *M. tuberculosis* and *M. bovis* [21, 22]. Guinea pigs are used as a sensitive in vivo culture system for tubercle bacilli [21], in testing of vaccines and drugs for the prevention and treatment of tuberculosis [26, 27], and for study of mycobacterial virulence [21, 28] and host resistance [5, 21]. Exposure of guinea pigs to low numbers of tubercle bacilli by aerosol results in pulmonary disease similar to that observed in humans; exposure to larger numbers of bacilli or exposure by other routes leads to rapid dissemination of disease and death [27, 29].

Recently, mice have been used as an animal model of tuberculosis, primarily for the study of antitubercular immunity [20, 30–33]. The advantages of the mouse model are the size and availability of the animals and the wide range of immunologic reagents available for studies in mice. However, mice are relatively resistant to tuberculosis and rarely develop disseminated disease as a result of non-intravenous exposure [20]. The lesions that develop in infected mice are well-demarcated granulomas or chronic fibrotic pneumonia [33]; liquefactive necrosis and cavity development rarely occur. Therefore, the mouse model does not demonstrate the entire range of lesions seen in humans with tuberculosis [24].

The purpose of these experiments was to develop and characterize a model of *M. bovis* infection in swine and to determine the similarities and differences between the infection in swine and humans. A new experimental model would be particularly useful if the full spectrum of human tuberculosis were reproduced, including development of classical tubercles in the lungs and other tissues, presence of tubercles with liquefactive necrosis, disseminated tuberculosis, and tuberculous meningitis.

**Materials and Methods**

**Animals.** Thirty-six 4-week-old mixed-breed pigs were obtained from a commercial source. Animals were housed on raised decks in class III biocontainment facilities, and food and water was provided ad libitum. Pigs were acclimatized for 1 week before inoculation with *M. bovis*. Personnel in contact with *M. bovis*-infected pigs were full-face, high-efficiency particulate air-filtered respirators.

*M. bovis.* The mycobacterial inoculum was prepared from a recent bovine isolate of *M. bovis* [34] grown in Middlebrook’s 7H9 liquid medium with 10% vol/vol OADC (Difco, Detroit) for 28 days at 37°C. Cells were harvested by centrifugation, washed in PBS, and diluted to the appropriate cell density in 1 mL of PBS. Tubercle bacilli were enumerated by plate counting on Middlebrook’s 7H10 medium with 10% OADC (Difco).

**Experimental design.** An initial experiment was conducted to determine an appropriate dose of *M. bovis*, route of inoculation, and time required for development of lesions in inoculated pigs. A second experiment was done to further characterize the experimental model and to determine a dose of *M. bovis* that would consistently produce disease limited to the lungs and draining lymph nodes. The combined results of the experiments are presented.

In the first experiment, pigs were anesthetized by intramuscular injection of 50 mg of xylazine and 50 mg of Telazol (A. H. Robins, Richmond, VA). Six groups of 3 pigs each were inoculated with either $2.8 \times 10^9$ or $8.4 \times 10^9$ cfu of *M. bovis* by one of three routes: intravenous (iv) injection in the cranial vena cava, intratracheal (int) deposition, or deposition on the surface of the tonsils. Two pigs were maintained as uninfected controls. Pigs recovered from anesthesia and were observed daily for development of clinical signs. One pig from each experimental group and one control pig were euthanized at 60 days after inoculation. Pigs were euthanized earlier if severe clinical signs developed.

In the second experiment, pigs were anesthetized, and groups of 6 pigs were inoculated with $10^6$ or $10^3$ cfu of *M. bovis* in 5 mL of PBS by int inoculation. Four pigs were inoculated with 5 mL of sterile PBS as uninfected controls. Pigs recovered from anesthesia and were observed daily for the development of clinical signs. Three pigs from each experimental group and 2 control pigs were euthanized 30 days after inoculation and the remaining pigs in each group were euthanized at 45 days after inoculation.

**Necropsy and sample collection.** Pigs were anesthetized by intramuscular injection of 150 mg of xylazine and 150 mg of Telazol. Cerebrospinal fluid (CSF) was collected for culture before euthanasia by pentobarbital overdose. A thorough postmortem examination was done to detect the presence of macroscopic lesions. Tissue specimens were collected and processed individually for histopathologic analysis and were pooled into groups, by body region, and frozen at $-80^\circ$C for culture. The body regions and tissues were as follows: brain; head (tonsil and mandibular, parotid, retropharyngeal, and deep cervical lymph nodes); thorax (lung and tracheobronchial and mediastinal lymph nodes); abdomen (spleen, liver, kidney, adrenal gland, and mesenteric, hepatic, and medial iliac lymph nodes); and peripheral lymph nodes (superficial cervical, subiliac, and deep popliteal lymph nodes).

**Culture.** Tissues were thawed and processed for culture by methods described previously [34, 35].

**Histopathologic examination.** Individual tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin-eosin by use of standard procedures. An additional section of each tissue was stained by a Ziehl-Neelsen staining procedure to detect the presence of acid-fast organisms [36].

**Results**

**Clinical signs.** Pigs given $2.8 \times 10^8$ cfu of *M. bovis* iv developed respiratory distress within 2 weeks after challenge. iv-infected pigs had elevated body temperatures (>40°C), had an increased respiratory rate, became cyanotic with mild exertion, coughed, and were euthanized 17 days after inoculation (1 pig) and 22 days after inoculation (2 pigs). One pig given $8.4 \times 10^4$ *M. bovis* int developed severe respiratory distress
and was euthanized 38 days after inoculation. Other pigs given 10^4 M. bovis iv, and those given either 10^6 or 10^8 M. bovis int, developed less severe signs of respiratory distress 30–60 days after challenge. Decreased appetite and weight loss were observed in these pigs. Clinical signs were not observed in pigs given 10^4 or 10^8 M. bovis by the tonsillar route or pigs given 10^2 or 10^3 M. bovis int.

**Culture.** At necropsy, M. bovis was isolated from one or more tissues from each pig inoculated with M. bovis. Mycobacteria were not isolated from control pigs. In pigs given 10^6 or 10^8 M. bovis by any route, mycobacteria were isolated from tissues of the head, thorax, abdomen, and peripheral lymph nodes with about the same frequency (table 1). Multiple tissue groups were culture-positive in 16 of 18 pigs, indicating the presence of disseminated infection. In addition, M. bovis was isolated from the brain or CSF of 7 of 18 pigs. M. bovis was isolated from the thoracic tissues of 11 of 12 pigs inoculated with 10^6 or 10^8 M. bovis int and sporadically from other groups of tissues (table 1).

**Gross lesions.** In pigs that developed disseminated tuberculosis, granulomas were detected at necropsy in the lungs, liver, spleen, peritoneum, and multiple lymph nodes. In pigs with disseminated disease or disease localized to the thorax, lung lesions varied from diffuse consolidation to multiple discrete granulomas. Fibrinous and granulomatous pleuritis were common.

**Microscopic lesions.** Lesions of tuberculosis were detected in each pig inoculated with M. bovis (table 2). In pigs given 10^4 or 10^8 M. bovis by any route, lesions were often widespread and involved most lymph nodes and tissues sampled. Lesions in pigs inoculated iv and those given 10^4 M. bovis by iv, int, or tonsillar routes were larger and more numerous than in pigs given 10^4 M. bovis by int or tonsillar routes. In contrast, lesions were limited to the lungs and tracheobronchial lymph nodes of 9 of 12 pigs given 10^2 or 10^3 M. bovis int; 3 pigs had small granulomas in the liver or hepatic or mesenteric lymph nodes in addition to thoracic lesions. The histologic character of the lesions was similar in pigs inoculated with each dose and by each route.

Early lesions (17–22 days after inoculation) consisted of multifocal areas of coagulative necrosis surrounded by macrophages, multinucleated giant cells, and smaller numbers of lymphocytes. Intracellular acid-fast organisms were abundant in these lesions (>50 organisms/40x field).

Pigs euthanized at 30 days after challenge had tubercles of various sizes in multiple organs. These tubercles were more organized than were lesions at 17–22 days and consisted of central areas of coagulative to caseous necrosis surrounded by a mantle of macrophages, epithelioid cells, and lymphocytes. Multinucleated giant cells were variably present at the periphery of the necrotic areas. The necrotic debris often contained significant numbers of neutrophils, and mineralization of the necrotic debris was seen in some granulomas. Fibrosis at the periphery of the tubercles was minimal. Acid-fast bacilli were rare in these lesions (<1 organism/40x field).

Tubercles observed in pigs euthanized at 45–60 days after inoculation were of two types. The most commonly observed type of tubercle was well-circumscribed and characterized by

<table>
<thead>
<tr>
<th>Group (route, inoculum)</th>
<th>Time sampled (days after inoculation)</th>
<th>Culture results</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Brain/CSF</td>
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<td></td>
<td>10^8</td>
<td>30–38</td>
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<tr>
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**NOTE.** Data are no. of pigs positive/no. tested. CSF, cerebrospinal fluid.
Table 2. Histologic analysis of tissues from pigs inoculated with *M. bovis* by intravenous, intratracheal, or tonsillar routes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Tissue</th>
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<th>Parotid LN</th>
<th>Retropharyngeal LN</th>
<th>Cervical LN</th>
<th>Lymph Nodes</th>
<th>Splenic Lymph Nodes</th>
<th>Hepatic Lymph Nodes</th>
<th>Iliac Lymph Nodes</th>
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NOTE. Data are no. of pigs with lesions of tuberculosis/no. tested. LN = lymph node. Peripheral LN = superficial cervical, subiliac, and deep popliteal lymph nodes. Lungs & LN = lungs and tracheobronchial and/or mediastinal lymph nodes.

Central necrosis with or without mineralization and neutrophils, a mantle of epithelioid cells and giant cells, and a thin collar of lymphocytes and fibrosis at the periphery (figure 1A). Acid-fast bacteria were rarely observed in these well-organized tubercles. Other tubercles were large expansive lesions with extensive caseation and liquefied necrotic debris. Few epithelioid cells were observed at the periphery of these tubercles. Lymphocytes were variably present surrounding the areas of necrosis. Large numbers of extracellular acid-fast bacilli (>100 organisms/40× field) were present in the liquefied necrotic material. The two types of tubercles were often found in close proximity within a tissue. Liquefied, uncontrolled tubercles were common in pigs with disseminated tuberculosis and rare in pigs inoculated with $10^2$ or $10^3$ *M. bovis* int.

Lesions were detected in the lungs of pigs inoculated by each route and with each dose of *M. bovis*. Lung lesions were characterized by multifocal, coalescing to diffuse granulomatous pneumonitis and pleuritis (figure 1B). Lung tissue adjacent to the granulomas was variably involved with an interstitial mononuclear cell infiltrate and macrophages in the alveoli. Exudate was variably present in airways, with macrophages and neutrophils being the predominant cells. Cellular thrombi containing macrophages and granulomas within the vessel wall were commonly observed in pulmonary blood vessels and lymphatics (figure 1C). Acid-fast bacteria were observed within macrophages in thrombi, bronchi, bronchioles, and alveoli. Lesions were variably distributed around airways and around blood vessels, suggesting dissemination of the infection via the airways and hematogenously.

Three of 6 pigs given *M. bovis* iv had lesions in the meninges, and *M. bovis* was isolated from the brain tissue or CSF from these pigs. Tuberculous granulomas were present within the wall of blood vessels in the meninges of the brain (figure 2A). The cellular infiltrate consisted of macrophages and a small number of lymphocytes. Necrosis was variably present within the vascular granulomas. One pig given $10^4$ *M. bovis* iv had a large subarachnoid granuloma that extended into the brain parenchyma (figure 2B). Acid-fast bacteria were identified in some meningeal granulomas.

### Discussion

In this study, swine were infected with *M. bovis* when challenged by iv, int, or tonsillar routes. Pigs given $10^6$ or $10^5$ *M. bovis* iv or int developed tubercles in multiple organs and lymph nodes, indicating that bacteremia occurred, with widespread dissemination of the organism. The disseminated disease in swine is similar in lesion distribution and character to that seen in extrapulmonary or disseminated tuberculosis in humans [37–41].

Challenge of swine with $10^5$ or $10^3$ *M. bovis* by the int route consistently produced lesions that were localized to the lungs and draining lymph nodes. Lesions were present within 30 days after exposure. Tubercles were generally well-organized and...
contained few acid-fast organisms; expansive, liquefied tubercles with extracellular *M. bovis* were uncommon. The histologic character of the pulmonary lesions observed is characteristic of well-controlled tubercles found in the lungs and draining lymph nodes of immunocompetent adults who develop primary tuberculosis [37, 38, 40]. The well-organized granulomas that were common in these pigs may have eventually healed or may have, in some cases, progressed to liquefied lesions with luxuriant mycobacterial growth.

Histopathologic analysis and culture were used to determine the extent of disease in pigs inoculated with *M. bovis*. The two techniques were generally in agreement; that is, presence of lesions within a tissue usually correlated with isolation of *M. bovis* from the corresponding group of tissues. However, in a number of cases, *M. bovis* was isolated from a group of tissues that were not found to contain lesions and vice versa. This probably results from differences in tissue sampling protocols for culture and histopathologic analysis, differences in sensitivity of the two detection methods, and presence of mycobacteria in tissues before development of detectable lesions.

Presence of well-organized controlled tubercles and uncontrolled liquefied tubercles in the same tissue is characteristic of tuberculosis in humans and rabbits [18, 24, 42]. Occurrence of the two types of tubercles within the same animal and tissue suggests that the host’s response to the organism and ability to control the infection involve local as well as systemic factors [24, 42]. Local factors, such as production of cytokines (e.g., interferon-γ), may result in activation of macrophages at the site of infection, with subsequent control of mycobacterial replication. Lack of activated macrophages at the local site of infection may lead to extensive replication of the mycobacteria within nonactivated macrophages and extension of the lesions.

Extensive uncontrolled tubercles with liquefaction of the caseous necrotic debris and luxuriant extracellular growth of
M. bovis were observed in lungs, lymph nodes, and other organs of infected pigs. The progression of tubercles to liquefaction, extracellular growth of mycobacteria, and cavitation are thought to be crucial events in the pathogenesis and spread of pulmonary tuberculosis in humans [24, 25, 42]. Liquefaction of the caseous necrotic material provides a favorable growth environment for the mycobacteria and leads to rapid extracellular growth. Delayed-type hypersensitivity to the large concentration of mycobacteria and their byproducts is thought to be important in the extensive tissue damage that occurs in liquefied tubercles [43]. Proteinases, nucleases, and lipases from macrophages also contribute to the liquefaction of the caseum [25]. Rupture of such a liquefied focus into an airway can lead to spread of the infection within the lung and into the environment [18, 42].

Meningitis was observed in 3 pigs given M. bovis iv. The lesions were characterized by granulomatous vasculitis and, in one case, formation of a subarachnoid granuloma with extension into the brain parenchyma. The pathogenesis of the granulomatous vasculitis is not clear but likely occurs secondary to bacteremia, with lodging of mycobacteria within the vasa vasorum or adventitial vessels. In most cases, the endothelium of the affected vessel was elevated, but intact (figure 2), which suggests that the inflammatory process began within the adventitia or tunica media rather than the intima of the vessel. This is consistent with observations made in the meninges of humans with tuberculosis [44, 45]. Acute fulminant tuberculous meningitis, which is observed in a small proportion of humans, most often children or immunocompromised adults, was not found [44, 45]. Fulminant tuberculous meningitis is thought to occur following extension or rupture of a granuloma into the subarachnoid space. This was not observed in this study, but such a lesion may require additional time to develop.

M. bovis was isolated from the brain or CSF of pigs in which menigitis was detected and from an additional 4 pigs in which meningitis was not detected. It is likely that the frequency of tuberculous foci in the brain and meninges was underestimated during this study because of the relatively small area of the brain (4 sections/pig) examined microscopically. An animal model of tuberculous meningitis is not available and is of considerable interest for pathogenesis and treatment studies [44, 45]. The findings of this study indicate that a swine model may be useful for the study of tuberculous meningitis.

Infection with M. tuberculosis is the most common cause of human tuberculosis worldwide. However, infection of human beings with M. bovis is still common in areas of the world in which the disease is common in cattle and has been recognized as a significant infection in immigrants to the United States [46, 47]. M. bovis and M. tuberculosis are extremely closely related and are both members of the M. tuberculosis complex of organisms. Lesions produced in humans infected with M. bovis and M. tuberculosis are of a similar histologic character but differ somewhat in their tissue distribution. The primary route of infection with M. bovis is more commonly oral, as opposed to a respiratory route of infection for M. tuberculosis. This results in lesions more often in cervical and abdominal lymph nodes in M. bovis infections than in M. tuberculosis infections. However, the distinction is far from absolute; primary pulmonary M. bovis infections do occur, and respiratory tract disease was found in up to 89% of M. bovis infections described in the last 3 decades [47]. We chose to study M. bovis rather than M. tuberculosis infection in swine for the following reasons: Swine are naturally infected with each of these organisms, and the lesions produced are indistinguishable [10]. Therefore, it is likely that swine are equally susceptible to M. tuberculosis and M. bovis. Second, because these organisms are so closely related, many research studies in tuberculo-
sis are done with M. bovis. In fact, much of the work on the pathogenesis and prevention of tuberculosis has been done in animal models infected with M. bovis. Third, the USDA research laboratory where the animal studies were conducted had considerable experience with M. bovis infections of various species and had virulent strains that had been recently isolated to work with.

In conclusion, swine are an excellent model for pulmonary and disseminated tuberculosis in humans. The advantages of the model are that lesions develop in inoculated animals within 30 days and that the spectrum of lesions is similar to that seen in humans. The dose and route of inoculation of M. bovis can be modified to produce disseminated disease, as a model of tuberculosis in children and immunocompromised persons, or localized pulmonary infection, more typical of tuberculosis in immunocompetent adults. In future studies, swine will be challenged with M. bovis by aerosol to study events following exposure by a natural route. The swine model of tuberculosis provides unique opportunities to investigate the influence of factors associated with an increased incidence of human tuberculosis, such as drug and alcohol abuse [2–8], on the pathogenesis of this disease. Furthermore, the swine model of tuberculosis will be useful in pathogenesis studies and in evaluation of the efficacy of antimycobacterial drugs and vaccines.

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References

33. Dunn PL, North RJ. Virulence ranking of some Mycobacterium tuberculosis and Mycobacterium bovis strains according to their ability to multiply in the lungs, induce pathology, and cause mortality in mice. Infect Immun 1995;63:3428±37.