Pathogenecity Studies of Group III (Battey) Mycobacteria
from Pulmonary Lesions of Man*

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CASES OF PULMONARY DISEASE THAT have many of the features of tuberculosis, but in which Mycobacterium tuberculosis has not been demonstrated have become recognized with increasing frequency during the past decade. We refer to instances of infection with unclassified mycobacteria designated "Group III (Battey)" that have been reported from widely separated areas of the world. ("Battey" refers to the Battey State Hospital, Rome, Georgia, where human infections with Group III mycobacteria were first reported.) The following references contain detailed information regarding the many problems relative to the unclassified mycobacteria associated with granulomatous pulmonary diseases in man: Chapman, Crow and co-workers; Engbaek; Engbaek and Magnusson; Hauduroy; Lewis and co-workers; Pinner; Prather and co-workers; Runyon; and Youmans. Appended to many of these reports are pertinent bibliographies. Also of value is the material presented at the Fifteenth International Tuberculosis Conference.

The frequency of occurrence of infections with Group III mycobacteria in the United States is generally believed to be relatively low, although it frequently has been identified in the southeastern part of the United States, particularly in Georgia and Florida. In Florida, approximately 2 per cent of patients admitted to the state tuberculosis hospitals yield atypical mycobacteria, most of which are classified as nonchromogens (Battey).

The pulmonary disease entity associated with infection with Group III mycobacteria is recognized more often in men than in women, and more often in white than in colored persons. The majority of patients are over 50 years of age.

A significant feature of this infection is its apparent noncontagousness: even intimate family contacts remain uninfected. This is an important distinction between pulmonary infections due to Myco.tuberculosis and those due to this unclassified variety of the genus.

Group III mycobacteria in the sputum or other respiratory exudates and in pulmonary lesions excised at operation or necropsy are readily cultured by standard methods used to isolate and culture Myco.tuberculosis. When the physical and biologic aspects of an initially isolated Myco.tuberculosis culture are compared with those of a Battey culture, however, several dissimilarities become apparent. The more important of these include rate of growth, range of temperature tolerated, and chromogenesis. Lastly, the pathogenic potentials of these two species of mycobacteria, compared by using standard test animals such as guinea pigs, are markedly dissimilar.

As is well known, guinea pigs are highly susceptible to experimental infection by virulent human tubercle bacilli. A 0.001 mg. dose of virulent human tubercle bacilli in a guinea pig usually produces a progressive, irreversible, and eventually lethal infection. Conversely, guinea pigs are highly resistant to Battey mycobacteria. Aside from developing tuberculin allergy, guinea
pigs tolerate intraperitoneal inoculations of 1 mg. or more of Battey mycobacteria as though the organisms were completely innocent.

The failure of Group III mycobacteria isolated from patients with demonstrable respiratory disease to produce progressive experimental infection in guinea pigs has prompted some to question whether these microorganisms are, in fact, etiologically related to the morbid changes in the pulmonary parenchyma. However, it seems reasonable to us that when tubercle bacilli cannot be obtained from a patient who has a tuberculosis-like pulmonary disease, but Group III mycobacteria can be obtained repeatedly, one may indict the latter organisms provisionally as contributory to the patient's illness.

The experimental demonstration that Group III mycobacteria are capable of impressive pathogenesis in a suitable host should provide at least indirect evidence that these bacteria may be pathogenic for man. The purpose of this report is to present experimental results which support this hypothesis.

**Animal Experiments**

The experimental studies, started about one year ago, were restricted to strains of Group III (Battey) mycobacteria. Cultures used were obtained from different persons, each of whom had been hospitalized because of pulmonary disease of some duration. If the assumption that these mycobacteria were sufficiently pathogenic for human beings to produce demonstrable pulmonary disease was valid, it seemed likely that the bacteria would possess potential pathogenicity for certain animals other than man.

**Hamsters**—The use of hamsters in experimental studies with mycobacteria has been reported by Griffith; Griffith and Pagel; Steenken and Wagley; Dennis and co-workers; Durr and co-workers; and Binford. Following a procedure essentially like that used by Binford in studies of the mycobacteria of human leprosy, a large group of golden Syrian hamsters (*Cricetus auratus*) were inoculated with a strain of Group III mycobacteria. The bacteria, isolated from the sputum and lungs before and after a patient's death, were designated "Strickland."

A suspension of bacterial cells was prepared in saline solution buffered with phosphate and divided into two portions. One portion was autoclaved twice at a pressure of 15 pounds for 25 minutes; the second portion was not heated but was protected from extraneous factors that might be injurious to the bacterial cells.

Each of the bacterial suspensions was used to inoculate a separate group of hamsters. The hamsters that received the heat-killed bacteria were considered the control group.

Inoculation of the animals was accomplished under sodium pentobarbital anesthesia. The anesthetic solution, containing 60 mg. of sodium pentobarbital per ml., was administered intraperitoneally. Dosage for hamsters weighing 100 gm. or more was computed on the basis of 0.01 ml. per gm. of body weight. All the animals in each group received the bacterial inoculum in the substance of each testis and under the dermis of the concha of each ear. The dose of bacterial cells injected into each testis was 0.001 mg., wet weight, and that injected into each concha was 0.0005 mg., wet weight. Twenty hamsters received heat-killed bacteria, and 46 received viable bacteria.

The experiment was terminated after 150 days.

**Results**

No signs of pathogenesis were observed grossly or microscopically in hamsters comprising the control group (Fig. 1A). In hamsters inoculated with viable, unheated, bacterial suspensions, gross and microscopic lesions were seen in the testes. In addition, oval, raised, firm nodules developed under

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*This and other cultures used in our studies were kindly furnished by Frank P. Dunbar, Chief of Laboratories, Southwest Florida Tuberculosis Hospital, Tampa, Florida.*
the dermis at the sites of inoculation in the conchae of the ears.

Microscopically, the morbid process in the testes was revealed in the early stages as a vigorous, essentially monocytic, or histiocytic, interstitial cellular response (Fig. 1B). In the later stages, epithelioid forms predominated. Lesions observed months after the infection had been established revealed limited necrobiotic and other retrogressive changes. However, caseation necrosis was minimal, and deposits of calcium were not seen. Associated with the changes mentioned was the presence of many intracellular, acid-fast bacilli. From the infected testes, we obtained cultures of mycobacteria similar to those from which the inoculum was prepared.

Other features of the testicular reactions, observed 150 days after the animals were inoculated, included progressive atrophy of the seminiferous tubules and the presence of laminated basophilic objects resembling so-called Schaumann bodies.* We observed few Langhans' cells.

In the concha of the ear, mycobacteria were not observed in the peripheral nerves despite their presence, sometimes in large numbers, in the granulomatous elements of the subepidermal zone (Fig. 2).

Also, in many of the hamsters, secondary focal lesions occurred in the liver, spleen,

*An account of the experimental production of Schaumann bodies in the golden hamster by certain microbial agents has been published recently by Okudaira and co-workers.*

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**Figure 1A (upper):** Testis of hamster killed 115 days after receiving intratesticular inoculation of heat-killed mycobacteria (Battey). Note the normal hexagonal design of the individual seminiferous tubules and absence of interstitial changes (hematoxylin and eosin; x55). **Figure 1B (lower):** Testis of a hamster which died 69 days after receiving intratesticular inoculation of viable mycobacteria (Battey). The lesion is characterized by marked interstitial proliferative reaction with various degrees of retrogressive changes in parenchymal cells of the seminiferous tubules. Acid-fast bacilli are moderately numerous (hematoxylin and eosin; x55).
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FIGURE 2 (upper): Cross section of granulomatous nodule in the concha of ear of a hamster. Animal had been inoculated with viable mycobacteria (Battey) 118 days prior to death. Reaction extends from subepidermal zone of the skin to cartilage; normal elements of appendage are replaced. Moderate number of Schaumann bodies are present. Acid-fast bacilli were demonstrated within the histiocytic cells (hematoxylin and eosin; x60). FIGURE 3 (lower): Section showing secondary foci in liver of hamster killed 115 days after receiving viable mycobacteria (Battey) intratesticularly. Schaumann bodies are present in each of nodules. Acid-fast bacilli were present and giant cells of Langhans' type occurred occasionally (hematoxylin and eosin; x70).
and certain abdominal lymph nodes (Figs. 3 and 4). Granulomatous lesions were not found in the lungs or kidneys. In one hamster, nephric amyloidosis was observed.

Repeated futile attempts to demonstrate hypersensitivity to tuberculin were made by the intradermal use of PPD-S and Battey PPD.† Undoubtedly, the capacity of hamsters to respond in delayed fashion is exceedingly small, as witnessed by the rather prolonged takes of homografts of skin.40,41 The failure of the golden Syrian hamster to reflect a state of delayed hypersensitivity to tuberculin was reported previously by Steenken and Wagley.42 Our observations were in agreement with their findings.

**Other Animals**

In addition to experiments with hamsters, studies were undertaken to determine the pathogenicity of Group III mycobacteria for rabbits and adult chickens. To

†The Battey PPD was kindly supplied by Dr. Lydia B. Edwards, Medical Director, Tuberculosis Program, Division of Special Health Services, Department of Health, Education and Welfare, Washington, D. C.

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**Figure 4:** Spleen of same hamster from which liver shown in Figure 3 was obtained. Lesions in spleen are characterized by diffuse areas of epithelioid cells with occasional Schaumann bodies. Moderate numbers of intracellular acid-fast bacilli are present. Neither necrosis nor encapsulation of the lesions is evident (hematoxylin and eosin; x165).

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As of the date, only a small portion of the material collected from these animals has been available for study and interpretation. Consequently, we are not prepared to present, at this time, more than casual observations concerning the ability of these mycobacteria to multiply and evidence other pathogenic features in experimentally inoculated rabbits and chickens.

Before being accepted for the experimental studies, all chickens were tested with avian tuberculin obtained from the U. S. Department of Agriculture. In no instance was a positive reaction elicited. Each chicken was inoculated intravenously or intracerebrally with 0.001 mg., wet weight, of bacterial cells.41 Each rabbit received 0.001 mg., wet weight, of the organisms intravenously. Chickens and rabbits were kept under observation for a minimum of six months; then the surviving animals were killed for necropsy.

**Results**

The rabbits gained weight and remained, throughout the period of the experiment,
in apparently good health. In none of the rabbits inoculated with the same strain of Group III mycobacteria used to inoculate the hamsters previously mentioned were lesions found at the time of death, 280 days after inoculation. In contrast to the marked resistance of the rabbits to Group III mycobacteria, various degrees of susceptibility were manifest in the chickens. Many chickens experienced progressive loss of weight, with marked atrophy of the muscles of the breast. A general weakening of some of the birds was a notable feature. However, results varied; they suggest that chickens of heterogeneous origin may differ in their responses to Group III mycobacteria.

In chickens inoculated intravenously, lesions occurred most often in the spleen and liver and contained few to many acid-fast bacilli. In some instances, the disease in these organs was destructively progressive and characterized by few to many microscopic focal lesions. Occasionally, the lesions almost replaced the entire substance of the spleen. In other instances, lesions in the spleen and liver were microscopic and non-caseating. Lesions containing various numbers of acid-fast bacilli were sometimes found in the marrow of the femur.

In chickens inoculated intracerebrally, lesions containing mycobacteria developed in the cerebral and meningeal tissues. Hematogenous extension of the infection from the brain to the liver and spleen was evidenced by the presence of granulomatous lesions in these organs.

It was our impression that the relative numbers of bacteria observed in lesions induced experimentally in chickens with Group III mycobacteria were much fewer than the numbers seen in lesions induced with freshly isolated, virulent Myco. avium. To determine if this is actually the case would require precise quantitative measurements.

COMMENTS

The observations recounted in this report support the view that mycobacteria other than Myco. tuberculosis are capable of producing chronic granulomatous pulmonary disease in human beings. Group III mycobacteria obtained by culture from such nontuberculous infections, though not pathogenic for guinea pigs, were capable, under the conditions of the experiments, of pronounced and apparently irreversible pathogenic action in hamsters after intratesticular inoculation. In addition, our observations indicate that Group III mycobacteria are capable of establishing a formidable morbid state in chickens, when infection is induced by intracerebral or by intravenous inoculation. These facts constitute impressive prima facie evidence incriminating these microorganisms as at least contributory factors in initiating morbid processes in human beings.

Since it appears that infection with Group III mycobacteria is recognized with increasing frequency and since these organisms are relatively resistant to commonly used chemotherapeutic substances, the search for more effective drugs should be intensified. Our results suggest that, in searching for such agents, intratesticularly inoculated golden Syrian hamsters may be useful.

Should subsequent evidence definitely establish that Group III mycobacteria are responsible for the pulmonary lesions with which they are associated, many questions will remain. These will need to be answered before we gain a clear understanding of how the infection is acquired. We should determine the natural source of the microorganism and whether animals other than man are naturally infected.

We believe the information obtained as a consequence of our experiments has important connotations for subsequent academic and clinical studies of human infections associated with unclassified mycobacteria. It is especially important to determine whether pathogenesis in human beings is dependent on certain preinfection conditioning factors. As more information concerning these bacteria is acquired, one may assume that official taxonomic recognition, indicating their precise relationship to other species of the genus, will eventual-
ly emerge for the so-called Battey mycobacteria.

**Summary**

To investigate the potential pathogenicity of Group III (Battey) mycobacteria, we experimentally infected golden Syrian hamsters (*Cricetus auratus*), chickens, and rabbits with bacteria obtained from several different patients. Anesthetized hamsters were inoculated intrathecally and in the subepidermal tissues of the conchae of the ears. Chickens received the bacterial inoculum intracerebrally or intravenously; rabbits, intravenously. Experiments were terminated at various times after two months.

Results demonstrated the pathogenicity of Group III mycobacteria for hamsters and adult chickens. For rabbits, the studies were inconclusive. In hamsters, the impressively consistent results were characterized by progressive, histiocytic, epithelioid, interstitial orchitis. Lesions frequently contained basophilic, laminated units resembling Schaumann bodies. Lesions in chickens occurred most often in the liver and spleen and were usually accompanied by progressive emaciation. In addition, infectious meningitis and cerebritis occurred in chickens inoculated intracerebrally.

The pathogenicity of Group III (Battey) mycobacteria, as demonstrated for hamsters and chickens, provides additional presumptive evidence of the disease-producing potential of these bacteria for human beings.

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**Résumé**


Les résultats mirent en évidence le pouvoir pathogène des bactéries du Groupe III pour les hamsters et les poulets adultes. Pour les lapins, les études ne furent pas concluantes. Chez les hamsters, les résultats nettement constants furent caractérisés par une orchite intersitielle progres- sive, histiocytique, épithélioid. Les lésions con- tenaient fréquemment des unités laminés baso- philie, ressemblant à des corps de Schaumann.

Les lésions chez les poulets atteignirent la plupart du temps le foie et la rate, et furent généralement accompagnées d’amaigrissement progressif. En outre une infection méningée et cérébrale se manifesta chez les poulets inoculés par voie intra- cérébrale.

Le pouvoir pathogène du Groupe III (Battey) démontré pour les hamsters et les poulets, four- nit une présomption supplémentaire de preuve du potentiel pathogène de ces bactéries pour les êtres humains.

**Zusammenfassung**

Zwecks Überprüfung der potentiellen Patho- genität der Mycobakterien der Gruppe III (Bat- tey) infizierten wir im Experiment sierische Goldhamster (*Cricetus auratus*), Hühner und


Die an Hamstern und Hühnern demonstrierte Pathogenität der Mycobakterien der Gruppe III (Battey) liefert weitere mutmaßliche Anhaltspunkte für die Krankheitsbewirkende Potenz dieser Erreger beim Menschen.

References


