Case Report Section

Isolation of Avian Tubercle Bacilli in Human Silicosis

ALFRED G. KARLSON, D.V.M., Ph.D., HOWARD A. ANDERSEN, M.D., F.C.C.P.
and GERALD M. NEEDHAM, Ph.D.
Rochester, Minnesota

Infection with Mycobacterium avium is common among poultry in many parts of the United States; such infection is also known to occur in swine and cattle.1 However, as far as we are able to determine, the isolation and unequivocal identification of avian tubercle bacilli from a human source have been recorded only once in this country.2 In the world literature available to us there are reports of approximately 25 cases in which the avian tubercle bacillus has been isolated from human sources and has been adequately identified, although infection of chickens, swine and cattle is known to exist in many countries.*

This report is made not only because it is unusual to find avian tubercle bacilli in material from human sources but also because evidence exists that the patient had silicosis. This is of special interest in view of the demonstration that relatively avirulent variants of tubercle bacilli may proliferate in experimental silicotic lesions of guinea pigs.7,9

Report of Case†

A 59-year-old white man was referred to the Mayo Clinic in October, 1950, because of increasing respiratory difficulty. Symptoms of pulmonary distress and edema of the lower extremities were so severe that he was hospitalized on arrival. Five years prior to admission the patient had started to work as a tool grinder. Two years later he had been hospitalized for pneumonia and pleurisy with effusion, and 600 ml. of fluid was withdrawn from the left pleural space. At that time he had been told his lungs were probably “dusted.” He had continued to work but had experienced periodic episodes of respiratory distress. A year prior to admission he had had “asthma” and shortness of breath and had been unable to work for about 6 weeks. At times it had been necessary to sleep supported by several pillows. In spite of this he had continued in his occupation until 2 weeks prior to admission, when dyspnea on the slightest exertion and edema of his legs forced him to rest.

Examination at the clinic revealed rales in each lung and edema of the feet, ankles and lower portions of the legs. A roentgenogram of the thorax revealed multiple patchy areas of infiltration in both lungs, a small amount of fluid in each pleural space and evidence of fibrosis in both bases (fig. 1). Review of the roentgenograms made elsewhere indicated a slight but definite progression of the pulmonary lesions for the past 3 years. A tuberculin test using 0.005 mg. of PPD gave no reaction. Microscopic examination of sputum failed to disclose acid-fast bacilli. Three specimens of sputum and two gastric washings were obtainable for culture of acid-fast bacilli.

A diagnosis of silicosis with cor pulmonale was made. The patient was treated with digitoxin and a diet containing 0.5 gm. of sodium. Great improvement took place; he lost 25 pounds of fluid in 7 days, and after 11 days of hospitalization he was dismissed. He returned home, where he died 2 weeks later of cardiac failure.

Section of Bacteriology, Section of Medicine, Mayo Clinic and Mayo Foundation.
The Mayo Foundation, Rochester, Minnesota, is part of the Graduate School of the University of Minnesota.

*Reviews of the reports on human infection with avian tubercle bacilli have been made by Feldman,1,2,4 Rich5 and Fontana.6
†This case has been briefly mentioned in a previously published abstract.7

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Necropsy disclosed chronic passive congestion of the lungs, liver and spleen. Adhesive pericarditis and bilateral pleuritis were noted. The lungs were severely anthracotic and several small cavities were adjacent to the anthracotic zones.

Microscopically, the anthracotic regions consisted of dense hyalinizing connective tissue with masses of pigment (fig. 2a). A few small foci of necrosis were present that contained a few acid-fast bacilli but the histopathologic picture was not typical of tuberculosis. When sections of pulmonary tissue were examined with polarized light, many collections of birefringent crystals resembling silica were seen (fig. 2b). Unfortunately, no material was available for study by incineration.

**Bacteriologic Examination**

Cultures for acid-fast bacilli were made on 5 successive days, using the aforementioned three specimens of sputum and two gastric washings. Four tubes of modified Löwenstein-Jensen medium were used for each specimen. As already indicated, no acid-fast bacilli were found in smears of the sputum; smears of the gastric contents were not made.

Colonies of acid-fast bacilli were obtained from each of the first four specimens, as shown in the table. The colonies were small, round, glistening and slightly yellowish or buff colored, which was recognized to be typical of avian tubercle bacilli. All the colonies on each tube in the successive isolations were identical in appearance. Organisms obtained on culture from one specimen were selected for tests of pathogenicity for chickens, rabbits and guinea pigs, and for in vitro tests of sensitivity to

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**FIGURE 1**: Roentgenogram at the time of admission showing patchy areas of infiltration and bilateral pleural effusion.

*We are indebted to Dr. D. G. Henderson, Director of Laboratories, Aultman Hospital, Canton, Ohio, for providing us with a report of the necropsy and for permission to examine histologic preparations.
Results of Cultures for Acid-fast Bacilli on 5 Successive Days

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Smear for acid-fast bacilli</th>
<th>Material</th>
<th>Results of culture*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative</td>
<td>Sputum</td>
<td>1 colony on 1 tube</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>Sputum</td>
<td>10 colonies per tube</td>
</tr>
<tr>
<td>3</td>
<td>Not done</td>
<td>Gastric washing</td>
<td>4 colonies per tube</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>Sputum</td>
<td>5 colonies per tube</td>
</tr>
<tr>
<td>5</td>
<td>Not done</td>
<td>Gastric washing</td>
<td>No growth in 8 weeks</td>
</tr>
</tbody>
</table>

*Average number of colonies per tube.

Drugs. Organisms grown from another specimen were subsequently used to repeat the tests for pathogenicity in animals.

Pathogenicity in Animals.—For the inoculation of animals, several colonies from the original culture were suspended in sterile water by rubbing a loopful of growth on the inside of the test tube. A uniform and stable suspension was readily formed, which is characteristic of avian tubercle bacilli. This suspension was diluted with sterile water to a density of one tenth that of tube no. 1 of McFarland's nephelometer. A portion of this suspension also was used to inoculate medium for in vitro tests for sensitivity to drugs as described later.

Two chickens, approximately 1 year old, were obtained from a flock known to have been free of avian tuberculosis for at least 2 years. They failed to react to either avian tuberculin or old tuberculin (OT) — when

**FIGURE 2A**

**FIGURE 2B**

*Figure 2a:* Section of lung showing dense fibrous areas with collections of black pigment. A few small necrotic foci contained a few acid-fast bacilli (hematoxylin and carbol fuchsin; × 100. *b:* Picture of same section taken with polarized light to show the birefringent crystals typical of silica (× 100).
tested 4 days prior to inoculation. Each received an intravenous injection of 1 ml. of the suspension. Each of two mature rabbits was given 1 ml. intravenously. Two guinea pigs received similar doses injected subcutaneously over the sternum.

The chickens died on the thirty-ninth and forty-first day respectively. In each, the spleen was about 10 times the normal size, the liver was enlarged and yellowish in color, and the kidneys were enlarged and congested. Smears from the spleen, liver and femoral marrow disclosed innumerable acid-fast bacilli. The rabbits died on the seventeenth and eighteenth day respectively, and each had enlargement of the spleen (six to eight times) and pulmonary congestion. Smears from the spleen showed innumerable acid-fast bacilli. The guinea pigs were tested on the eighty-sixth day with 0.1 ml. of a 1:100 dilution of OT and with avian tuberculin. The reaction to OT consisted of a firm erythematous zone about 6 mm. in diameter, whereas the reaction to avian tuberculin was a raised erythematous zone 25 mm. in diameter. When the guinea pigs were killed on the ninetieth day of infection, a small caseous lesion about 5 mm. in diameter was found in each at the site of injection, along with necrosis of an axillary lymph node. All other organs were grossly normal.

Histopathologic examination of various organs from the rabbits and chickens disclosed widespread tuberculous lesions containing large numbers of acid-fast bacilli. The organs of the guinea pigs were microscopically normal except for the sites of inoculation and the axillary lymph nodes, which presented circumscribed necrotic processes with many acid-fast bacilli. Cultures were made from the spleens of the chickens and rabbits and the site of inoculation of one guinea pig; colonies typical of avian tubercle bacilli eventually appeared in all. Organisms obtained on culture from the spleen of a rabbit produced fatal disease in two chickens and two rabbits but failed to cause any lesions in the internal organs of two guinea pigs.

The tests for pathogenicity in animals were repeated, using another of the original cultures from this patient. The procedures were the same as in the series just described. The rabbits died on the seventeenth and twenty-fourth day respectively, and the chickens died on the twenty-eighth and fifty-fourth day. The latter chicken was tested on the thirty-fifth day with 0.1 ml. of a 1:100 dilution of OT and 0.1 ml. of avian tuberculin, each given intracutaneously in a wattle. There was no reaction to the OT, whereas a 4+ reaction was recorded for the test with avian tuberculin. Smears from the enlarged spleens of the rabbits and chickens contained innumerable acid-fast bacilli.

The guinea pigs were tested on the thirty-fifth day with avian tuberculin and with OT as described for the first series. Only a slight reaction occurred to OT but the test with avian tuberculin produced a 4+ reaction with necrosis. The tuberculin tests were repeated on the seventy-fourth day. The tests with OT resulted in a slight reaction in one guinea pig and a doubtful reaction in the other. The avian tuberculin, however, caused erythematous raised zones 20 mm. in diameter, with necrosis at the centers.
In Vitro Sensitivity Tests.—A portion of the suspension used to inoculate the first series of animals was inoculated on three series of egg-yolk agar medium containing streptomycin, para-aminosalicylic acid and isoniazid respectively. It was found that the microorganisms were resistant to 10 but not to 50 micrograms of streptomycin, to more than 10 micrograms of para-aminosalicylic acid and to more than 40 micrograms of isoniazid per milliliter respectively. This relative resistance is characteristic of avian tubercle bacilli.

Comment

As already mentioned, although no material was available for incineration, examination of sections by polarized light did reveal innumerable birefringent crystals characteristic of silica. The diagnosis of silicosis is substantiated by the history, the roentgenographic appearance, the signs of cor pulmonale and the histopathologic appearance of the pulmonary lesions. We believe that the finding of avian tubercle bacilli is incidental and that these microorganisms played little part, if any, in the pulmonary fibrosis. It may be that the silicotic lesions provided a more favorable medium for growth of the avian tubercle bacilli, which normally are relatively avirulent for man. This suggestion is made in view of the aforementioned finding that avirulent variants of tubercle bacilli may proliferate in experimental silicosis in guinea pigs but fail to become established in normal guinea pigs.9 We have been unable to learn anything of the patient’s history with respect to the source of the microorganism. It is of interest to note that his home was in a rural community in that part of the United States where tuberculosis among chickens is most prevalent.1

Infection with the avian tubercle bacillus is so rarely reported in man that skepticism regarding its occurrence is justifiable. We agree with Rich4 that in the majority of reports the identification of avian tubercle bacilli is incomplete or is such as to cast doubt on the validity of the claim. We also agree with Rich that proof of the identity of avian tubercle bacilli must rest on pathogenicity for rabbits and chickens and failure of the microorganism to produce progressive disease in guinea pigs. In our case, these criteria have been met in each of two cultures isolated from the patient on different days. The animal inoculations were made with original cultures. The isolations were made in a laboratory where avian tubercle bacilli were not being handled.

SUMMARY

Acid-fast microorganisms with the cultural characteristics of avian tubercle bacilli were isolated from three specimens of sputum and a gastric washing from a patient with silicosis. These organisms were virulent for chickens and rabbits but failed to produce progressive disease in guinea pigs. The infected animals reacted slightly to old tuberculin but severely to avian tuberculin. Additional studies on one culture revealed it to be resistant in vitro to streptomycin, para-aminosalicylic acid and isoniazid, which is typical of avian tubercle bacilli.
The patient died of cardiac failure. Sections of lung revealed extensive pneumonoconiosis and fibrosis. Birefringent crystals typical of silica were seen in sections examined with polarized light. It is believed that the avian tubercle bacilli played little part, if any, in the production of pulmonary fibrosis and that these microorganisms were able to proliferate because of the pre-existing silicotic lesions in a host for which avian tubercle bacilli are normally relatively avirulent.

RESUMEN

Se aislaron microorganismos ácido-resistentes con las características de cultivo del germen aviar, de tres muestras de esputos y lavado gástrico de un enfermo de silicosis. Estos organismos fueron virulentos para las gallinas y los conejos pero no produjeron enfermedad progresiva en los cuyes. Los animales infectados reaccionaron ligeramente a la tuberculina antigua pero severamente a la tuberculina aviar. Los estudios adicionales de un cultivo revelaron que era resistente in vitro a la estreptomicina, al ácido paraminosalicílico y a la isoniaciada, lo cual es típico del bacilo aviar.

El enfermo falleció de insuficiencia cardiaca. Las secciones del pulmón revelaron extensa pneumonoconiosis y fibrosis. Se encontraron cristales birrefringentes típicos de silice en las secciones examinadas con luz polarizada. Se cree que el bacilo aviar desempeñó muy pequeño papel, si acaso alguno, en la producción de la fibrosis pulmonar y que estos microorganismos fueron capaces de proliferar a causa de las lesiones silicosas preexistentes en un huésped para el cual aviar es, normalmente, avirulento.

RESUME

Des micro-organismes acido-résistants ayant les caractères de culture des bacilles tuberculeux aviaires ont été isolés à partir de trois produits d'expectoration et de lavage gastrique chez un malade atteint de silicose. Ces microbes étaient virulents pour les poulets et les lapins, mais ne réalisèrent aucune affection évolutive chez les cobayes. Les animaux infectés réagirent faiblement à la tuberculine brute de Koch, et fortement à la tuberculine aviaire. Les études ultérieures d'une culture montrèrent que le bacille était résistant in vitro à la streptomycine, au P.A.S. et à l'isoniazide, ce qui est caractéristique du bacille tuberculeux aviaire.

Le malade mourut d'insuffisance cardiaque. Des coupes du poumon montrèrent une pneumonoconiose extensive et une fibrose. Des cristaux biréfringents de silice furent notés sur les coupes examinées à la lumière polarisante. Les auteurs pensent que le bacille aviaire ne joua qu'un faible rôle, si toutefois il en joua un, dans la production de fibrose pulmonaire. Pour eux ce sont les lésions silicotiques préexistantes qui permirent la végétabilité de ces bacilles.

REFERENCES

Trends in Tuberculosis Therapy

JACK D. COHEN, M.D.*

Boston, Massachusetts

The following case of pulmonary tuberculosis is reported because of its unusual features. In one person are encountered most of the current medical and surgical procedures as they have appeared over the past 10 years. This patient is still alive and well because of the propitious appearance of chemotherapeutic and antibiotic aids, with advances in thoracic surgery. The striking transition in the treatment of tuberculosis plus its various pitfalls and complications are demonstrated.

This is the history of a 36 year old woman who contracted her disease in 1942 at the age of 25, 20 months after the birth of her only child. There was no known contact or family history. An x-ray film taken at the onset of symptoms of cough, pleurisy and fever revealed minimal pulmonary tuberculosis. On being checked five months later at the local health unit, this had spread to far advanced bilateral disease. On May 1943, she entered the sanatorium.* Pneumothorax and bed rest was then the accepted treatment for fresh exudative progressive disease. Following negative bronchoscopy, artificial pneumothorax was instituted, first on the right and then on the left. She responded well, and in October 1944, after a year and a half of treatment, was discharged with the advice to maintain the bilateral collapse. A year later, in 1945, a large right pleural effusion appeared accompanied by fever and dyspnoea. Despite thoracentesis the fluid re-accumulated and it was necessary to abandon both the right

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*Channing Home for Tuberculosis, Boston, Mass.
*Visiting Physician, Channing Home, Cambridge City Hospital, Assistant in Medicine, Beth Israel Hospital.