Résumé On a comparé l'épreuve de floculation au sulfate de zinc avec les valeurs de la protéinémie dans 200 spécimens de sérum pathologique, pour pouvoir prédire la présence ou l'absence de paraprotéinémie. La corrélation était exacte dans 84.5% des cas. Par cette méthode, sept cas de paraprotéinémie sur 68 n'ont pas été décelés. On estime que la réaction de floculation au sulfate de zinc, associée au dosage des protéines sériques, constitue un moyen précieux de dépistage de la paraprotéinémie.

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CASE REPORTS

"Anonymous" Mycobacterial Infection Causing Disseminated Osteomyelitis and Skin Lesions

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"ANONYMOUS" mycobacteria have been reported as causing mainly pulmonary disease in adults[1-4] and cervical adenitis in children.[5-8] In contrast, there have been relatively few references to concomitant bone and skin involvement[9-12] or to bone lesions[13] with visceral manifestations.[14-16]

The purpose of this paper is to report the occurrence in a child of disseminated granulomatous skin and bone lesions from which a strain of Battey-type mycobacterium was isolated repeatedly and which appeared to be the etiological agent. Although *Staphylococcus pyogenes* was the only organism that could initially be isolated from bone fragments, it was only after antituberculous therapy had been instituted that definite improvement and healing of both types of lesions was observed.

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P.B., a 5½-year-old white girl, was admitted to St. Justine Hospital in March 1965 with a presumptive diagnosis of generalized osteomyelitis.

Ten months before admission, she had developed erythema and edema of the right forearm, left ankle and right heel. Her family physician referred her to the local hospital with a tentative diagnosis of rheumatic fever. According to the information obtained, the patient's temperature varied between 98° and 101° F. during the first week of hospitalization; her white blood cell count (WBC) was 13,000 with a predominance of lymphocytes, and her erythrocyte sedimentation rate (ESR) was 42 mm. Upon treatment with penicillin, acetylsalicylic acid and steroids, the inflammatory reaction quickly disappeared. Three weeks later, however, radiological evidence of osteomyelitis was noted and there was a coincidental appearance of cutaneous lesions. Steroid therapy was discontinued. The patient remained in hospital for a further three months and was then discharged with a diagnosis of acute osteomyelitis of the right forearm, left ankle and right foot. She was readmitted to the same hospital in December 1964, at which time improvement in the

TABLE I.—PATHOLOGICAL AND BACTERIOLOGICAL FINDINGS IN P.B., A 5½-YEAR-OLD WHITE FEMALE CHILD

Date	Specimen	Pathology	Bacteriology: Direct exam. (Ziehl)	Bacteriology: Culture	G.P. inoculation (90 days after inoculation)	Drug therapy
3-15-65	Cutaneous lesions, rt. leg, lt. forearm Pus from three locations	—	Negative	3 positive *Staph. pyogenes* types 3 C^{++++}, 55^{+++}, 71^{+++} 2 positive Battey-type mycobacteria	No TB lesions	None
3-19-65	Bone fragments, upper third lt. ulnar	Necrotic and fibrous tissue infiltrated with lymphocytes, plasma cells, eosinophils, rare polymorphonuclear cells and subacute osteomyelitis	Negative	Positive only for *Staph. pyogenes*	—	
4-23-54	Cutaneous lesions, pus	—	Positive	Neg. for staphylococci and mycobacteria	—	Nafcillin for 33 days
4-27-65	Cutaneous biopsy, rt. leg	Granuloma with giant cells (Langhans type) and polymorphonuclear cells; inflammatory exudate	—	—	—	—
6-21-65	Bone fragments, rt. radius	Chronic inflammatory reaction with predominance of plasma cells; no granulomatous lesions	Positive	Negative	No TB lesions	Streptomycin for 50 days Ethionamide for 33 days
7- 9-65	Cutaneous biopsy, 2nd lt. toe	Chronic and acute inflammatory reaction with plasma cells and a few multinucleated giant cells	—	—	—	Still on streptomycin and ethionamide
5-16-66	Bone fragments, 2nd-5th metacarpal left hand	Subacute inflammatory reaction with lymphocytes polymorphonuclear and giant cells	Positive	Positive for Battey-type mycobacteria	Not done	On ethionamide until 8-16-66
9-30-66	Bone fragments, rt. ext. malleolus	Chronic osteomyelitis, granulomatous lesion	Positive	Positive for Battey mycobacteria Postive for *Staph. pyogenes* types 52^{+++}, 80^{+++}	Not done	On lincomycin since 5-7-66

bone lesions was noted radiographically. No further details concerning her therapy or disease are available until she was referred to St. Justine Hospital on March 12, 1965.

On admission the patient appeared well developed and well nourished, with no evidence of toxicity or acute distress. She was afebrile. On physical examination, the only positive clinical findings were skin pallor, a soft systolic cardiac murmur grade II/IV and generalized skin lesions. On both lower extremities, right hip and right arm the lesions were deep-seated, surrounded by an inflammatory area 1 to 3 cm. in diameter, moist and crusted.

Laboratory Findings

On admission, her hematological values were: hemoglobin 9.5 g. per 100 ml.; red blood cell count 3.8 million per c.mm. with 0.8% reticulocytes; white blood cell count 20,000 with 74% polymorphonuclear neutrophils and 4% band cells; and corrected erythrocyte sedimentation rate 24 mm. in one hour. The results of protein electrophoresis (in g. per 100 ml.) were: total protein 8.4, albumin 3.19, alpha$_1$ globulin 0.58, alpha$_2$ globulin 1.23, beta globulin 1.17 and gamma globulin 2.23.

Details of bacteriological and pathological findings are shown in Table I. After incubation of pus

for three weeks at 37° C. on Lowenstein's medium, smooth moist colonies were obtained, consisting of pleomorphic acid-fast organisms. The bright-yellow colonies grown in the dark did not change in colour when they were incubated further under light exposure. On subculture, bacterial growth occurred within 14 days at 37° C., and in 21 days or more at room temperature; however, no growth occurred at 50° C. The colonies dispersed readily in a liquid medium, and growth was observed in a thioglycollate medium in 14 days; no cord formation could be detected. Various-sized inocula of the strain grown in Dubos medium (Tween-80 and albumin) for six days failed to produce any definite disease in guinea-pigs, Swiss white mice and hens. The catalase reaction was strongly positive.

Identification of the organism by more refined methods including phage typing, enzyme studies and immunodiffusion was accomplished through the courtesy of Dr. E. Mankiewicz of the Royal Edward Hospital Laboratory in Montreal. It was reported as being a Battey organism, highly resistant to the three major antituberculous chemotherapeutic agents tested (streptomycin, isoniazid, para-aminosalicylic acid), but sensitive to ethionamide.

Coagulase-positive *Staphylococcus pyogenes* isolated from cultures obtained at several sites, including blood, showed the same phage type and the same sensitivity pattern to antibiotics. Mycological cultures gave negative results.

Intradermal tests were made repeatedly with commercial PPD (0.001 mg. per ml.), histoplasmin (1/100) and blastomycin (1/100), as well as with tuberculin using the Tine test; all proved negative. Tests made in June 1965 with additional mycobacterial antigens (0.0001 mg. per ml.), obtained through the courtesy of Dr. Lydia B. Edwards of the United States Public Health Service, produced indurations of the following diameters: P.P.D. – S = 0 mm.; P.P.D. – G = 12 mm.; P.P.D. – Y = 12 mm.; and P.P.D. – B = 15 mm.

Radiological Studies

Repeated chest radiographs showed no evidence of any pulmonary infectious process, either past or present.

Radiological examination of the extremities on admission showed osteolytic lesions, without sequestra, surrounded by mild, diffuse sclerosis. There were a few lamellar-type periosteal detachments in the upper extremities. Cutaneous nodules were present, particularly on the lower right and upper left extremities. These nodules had no relation to the bone lesions.

The osteolytic lesions were present in several bones of the extremities. There was almost total involvement of the right humerus, right ulna, first and second metacarpals, fourth left metacarpal, fourth right metatarsal and first and third left metatarsals. There were also multiple lesions of both tibias, both radii and the left ulna. There were individual lesions of the right fibula, right radius, left humeral metaphysis and cuboid. The following bones did not seem to be involved: cranium, spine, thorax, pelvis and right fibula.

Course of the Illness and Treatment

Three days after admission and following curettage of the bone lesions for diagnostic purposes, the general condition of the child suddenly deteriorated and her temperature rose to 104° F. Nafcillin therapy was started immediately and continued in a dosage of 6 g. daily intravenously for the first nine days, followed by combined oral nafcillin (6 g. daily) and probenecid for a further period of 47 days. The patient's clinical condition improved considerably and the inflammatory reaction surrounding the cutaneous lesions began to disappear. The bone lesions, however, increased and the appearance of new inflammatory foci was noted on the radiographs. The child complained of severe pain, particularly in the lower extremities, and was unable to walk.

At the beginning of May 1965, when consideration was given to the possible role played by anonymous mycobacteria in her disease, antituberculous therapy was started: streptomycin 40 mg. per kg. per day and isoniazid (INH) 20 mg. per kg. per day. Two weeks later, when the organism was reported as INH-resistant, INH was replaced by ethionamide 750 mg. per day, which was continued until April 1966.

As early as one week after initiation of antituberculous therapy, the child ceased complaining of pain in her extremities. Within two months there was marked improvement in the appearance of the cutaneous lesions, and radiological examination failed to detect any new osseous inflammatory foci. The smallest and most recently discovered lesions had disappeared and most of the others had decreased in size. The patient was discharged July 17, 1965, on ethionamide therapy.

When the patient was readmitted in September 1965 for re-evaluation, only atrophic and scar tissue were present at the site of the former skin lesions, and radiographs of the involved bones again showed marked improvement. In December 1965, complete healing of the various skin and bone lesions was observed. Prolonged ethionamide therapy had produced no clinical side effects and no detectable alterations of liver and kidney function tests. The child had gained weight and could walk satisfactorily. Despite the clinical cure and healing of the bone and cutaneous lesions, treatment was continued for an additional six months with periodic examinations for signs of toxicity.

The child was readmitted in April 1966 with swelling and tenderness over the left hand. Radiographs revealed that new lesions, localized mainly in the left metacarpal region, had appeared. Direct examination of bone fragments obtained by curettage of the lesions showed numerous acid-fast bacilli; culture of the fragments produced a strain of mycobacterium similar to the Battey-type organism originally isolated. However, it had lost its susceptibility to various phages and was resistant to

high concentrations of ethionamide, isoxyl (4-4 Diisoamyloxythiocarbanilide) and such other antibiotics as cycloserine (100 mg. per ml.), kanamycin (100 mg. per ml.) and lincomycin (2 mg. per ml.). As the first strain isolated a year previously had shown sensitivity to lincomycin, this antibiotic was selected as possibly the most suitable drug at this time in the patient's course.

During the nine months on lincomycin, there was improvement of the metacarpal lesions, but new inflammatory bone and skin foci appeared in the right malleolar region, at which time curettage was again required.

Although some lesions still persisted, all medication was discontinued in February 1967 and since then the child's general condition has remained excellent.

DISCUSSION

Considering the course of the illness with its insidious onset, low-grade fever and a normal neutrophil count, it is likely that the primary etiological agent in the child's disease was the anonymous mycobacterium and that *Staphylococcus pyogenes* appeared as a secondary invader for a short period. The radiological evolution provides additional evidence of the dominant role played by the mycobacterium; indeed the course was not that which is usually seen in staphylococcal osteomyelitis with its multiple foci at onset and the marked aggravation during antistaphylococcal therapy, absence of sequestra at all times and complete healing without sclerosis.

Our therapy was based on *in vitro* sensitivity tests. Like most other anonymous mycobacteria isolated from human infections, our strain appeared from the beginning to be highly resistant to the most commonly used antituberculostatic drugs. Response to ethionamide appeared favourable over a fairly long period of time without any noticeable side effects. Whether it should have been alternated with or given in association with another drug in order to delay the appearance of strain resistance needs further consideration. Other strains of mycobacteria have been reported sensitive to antibiotics such as kanamycin[1] and erythromycin.[7] The strain isolated from the lesions in this child had become resistant to ethionamide, although it did show sensitivity to commonly used antibiotics by the dilution method but at concentrations that could not safely be reached in the blood except with lincomycin.

In a search of the literature we could find only nine cases of mycobacterial infections in which bone lesions were reported; five[11, 13-16] and possibly six[9] of these were due to a Battey-type organism. Among the last group, four were children who had visceral manifestations with a fatal outcome. So far our patient has shown no visceral manifestations, and the evolution of the disease, although chronic, appears to be favourable.

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