Histologic Observations of Pleomorphic, Variably Acid-fast Bacteria in Scleroderma, Morphea, and Lichen Sclerosus et Atrophicus

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ABSTRACT: Variably acid-fast coccoid forms, suggestive of cell wall deficient forms of mycobacteria, were observed in the dermis in microscopic sections of skin from six patients with generalized scleroderma, 10 patients with localized scleroderma (morphea), and four patients with lichen sclerosus et atrophicus (LSA). These coccoid forms were found within the collagen bundles, around the adnexae (hair shafts, pilosebaceous units, eccrine glands), and less commonly around the blood vessels and nerves. These coccoid forms may be related to cocci and also to granular coccoid elements of corynebacteria-like coccobacilli, which, on occasion, can be cultured from the skin of these three diseases. The findings in this study support the three-decade old hypothesis concerning the constant association of pleomorphic acid-fast bacteria with scleroderma. The study also suggests that closely related diseases, such as morphea and LSA, are also associated with the presence of similar appearing microbes.

The association of variably acid-fast, highly pleomorphic bacteria with generalized scleroderma (progressive systemic sclerosis) and morphea (localized or circumscribed scleroderma) has been proposed by Wuerthele-Caspe Livingston et al in a series of reports (1947–1972)[1–3]; by Delmotte and van der Meiren in 1953[4]; and by Cantwell et al in a series of reports (1966–1980).[5–11] Similar, variably acid-fast bacteria and corynebacteria-like microbes have also been implicated in the pathogenesis of neoplastic,[2,3,12,13] lymphoproliferative,[14,15] and certain other "collagen diseases"[16–19] by a number of different investigators.

Although there is no unified and orderly classification of scleroderma,[20] the disease may occur as localized skin lesions (morphea) or as generalized scleroderma with or without involvement of other organs. Both forms of cutaneous scleroderma have a similar histopathologic picture.[21] The classification of lichen sclerosus et atrophicus (LSA) is still controversial.[22] Authorities, such as Goltz,[23] Winkelmann,[23] Abulafia,[23] and Jablonska[23] consider LSA to be related to morphea. This proposed relationship has been strengthened by Uitto et al[24] who studied 10 patients with skin lesions that were clinically consistent with the diagnosis of both morphea and LSA. Histopathologic changes of both morphea and LSA also were present in all cases.

This report presents microphotographic evidence of microbial forms in vivo in microscopic sections of scleroderma, morphea, and LSA. The previous results of bacteriologic culture of the skin and blood of scleroderma, as well as the results of animal inoculation studies with scleroderma isolates, have been reported elsewhere[1,2,3,5–10]; however, several microphotographs of certain cocci and corynebacteria-like coccobacilli that have been isolated from cutaneous lesions of scleroderma and morphea will be presented in order to compare the in vitro and in vivo appearances of these microbes. Wuerthele-Caspe Livingston et al,[2,3] Mattman,[25] and Cantwell et al[9–11] have all suggested that the pleomorphic microbes (cocci, acid-fast and non-acid-fast bacilli, filamentous forms, "granules," and "large bodies") associated with scleroderma may be cell-wall deficient L forms of mycobacteria, corynebacteria, or other closely allied actinomycetes.

This report will suggest that all three diseases (scleroderma, morphea, and LSA) are associated with variably acid-fast bacteria in vivo.

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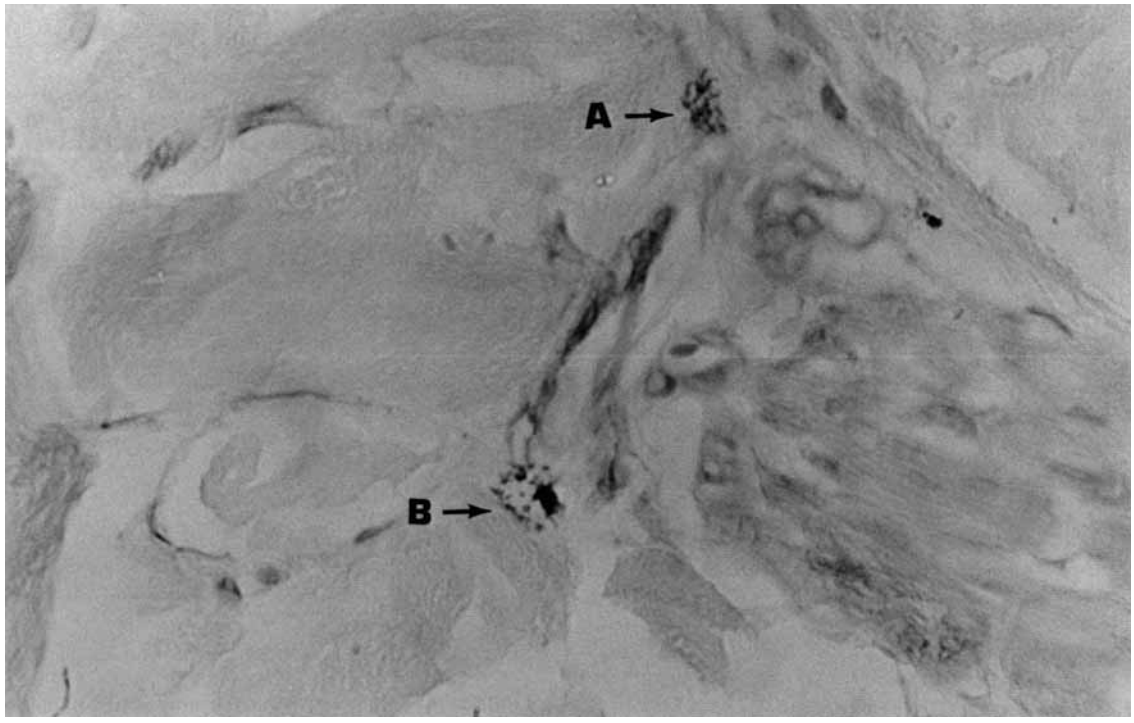

FIG. 1. Generalized scleroderma. Two foci (arrows) of purple-stained coccoid forms in the deep dermis. (Case 1; Fite stain, original magnification × 400.)

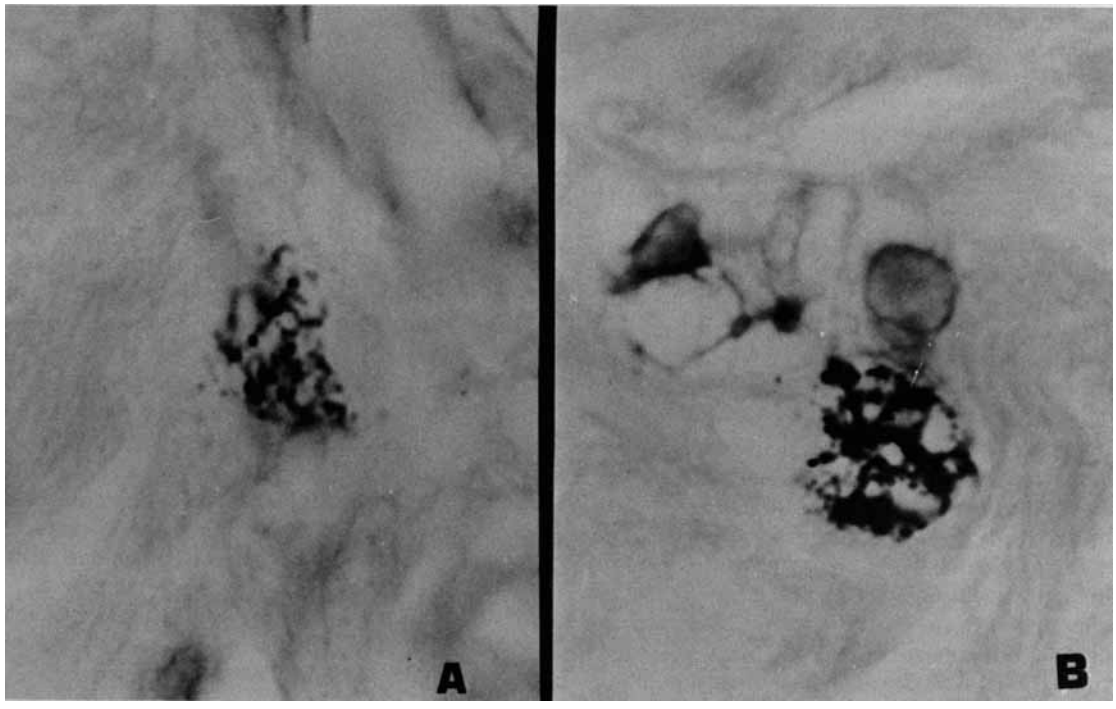

FIG. 2A. Generalized scleroderma. Same section as Fig. 1 showing coccoid forms in Focus A at a higher magnification. 2B an additional focus of variably sized, purple-stained coccoid forms in the deep dermis. (Case 1; Fite stain, original magnification × 1000, in oil.)

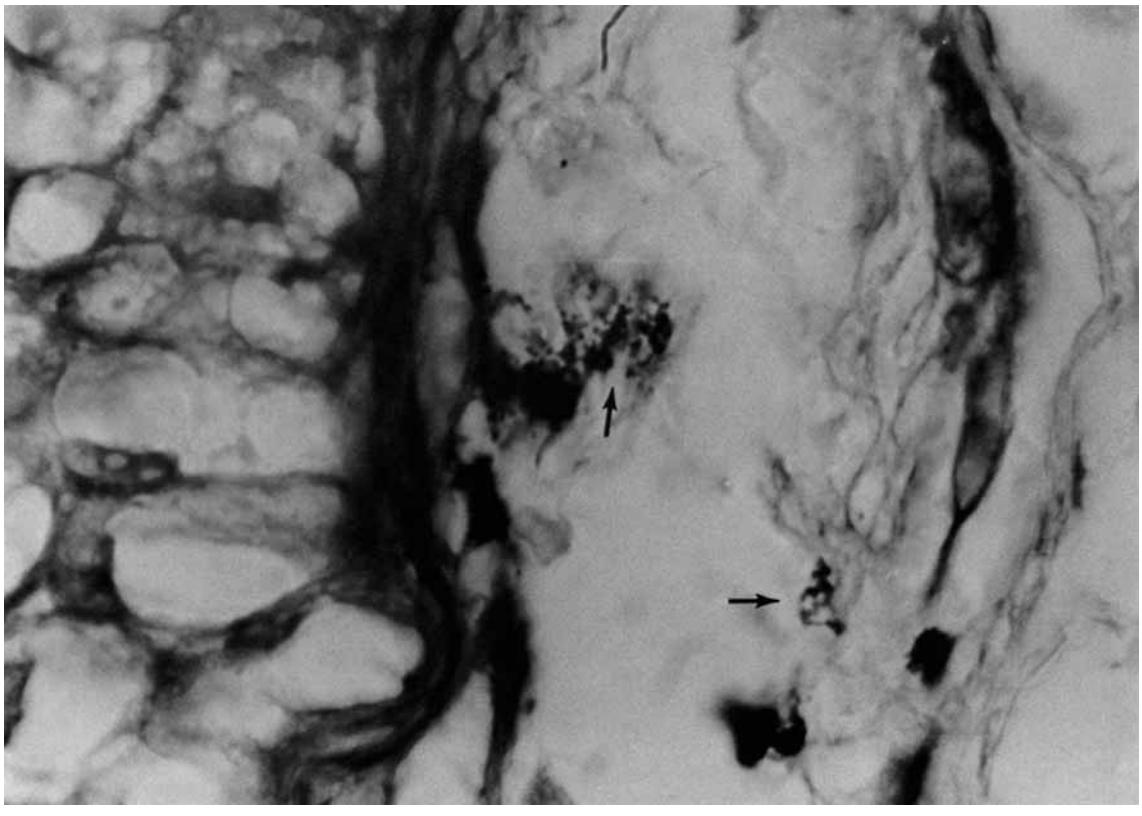

FIG. 3. Generalized scleroderma. Two foci (*arrows*) showing purple-stained coccoid forms near a hair shaft in the mid-dermis. (Case 2; Fite-Faraco stain, original magnification × 1000, in oil.)

Materials and Methods

The skin biopsy material from six previously unreported patients with generalized scleroderma was studied. There were five women and one man, ranging in age from 18 to 70 years. The 10 patients with morphea consisted of six men and four women, ranging in age from 9 to 64 years. The four patients with LSA (three women; one man) ranged in age from 14 to 80 years. All 20 patients had a clear-cut clinical diagnosis confirmed by a pathologist's report of a skin biopsy specimen. The pertinent clinical details of the seven cases chosen to illustrate microbes in vivo have been tabulated in Table 1.

Bacteriologic examinations of skin biopsy material were not performed on Cases 1, 4, 5, and 7. Both *Staphylococcus epidermidis* and pleomorphic coccobacilli compatible with corynebacteria (diphtheroids) were cultured from multiple skin biopsy specimens from Case 2. Case 3 also yielded pleomorphic coccobacilli. Case 6 yielded a pleomorphic coccus consistent with *Micrococcus* sp. Recently, a highly pleomorphic microbe was cultured from skin biopsy material from a patient with generalized morphea. Peculiarly, this microbe vacillated in its morphologic appearance in culture and sub-culture between a *Staphyloccus epidermidis*-like microbe on solid media, such as blood agar, and a corynebacterial-like coccobacillus in liquid media, such as thioglycollate broth (unpublished data).

TABLE 1. *Clinical Information on Study Cases*

Case No.	Diagnosis	Sex	Age (yr)	Race	Site of Biopsy	Duration of Disease (yr)
1	Scleroderma	F	18	Black	Forearm	1
2	Scleroderma	M	50	White	Forearm	6
3	Scleroderma	F	49	White	Knee	7
4	Morphea	F	70	White	Hip	1
5	Morphea	F	53	White	Breast	1
6	Linear morphea	F	9	White	Leg	2
7	LSA	F	14	White	Shoulder	2

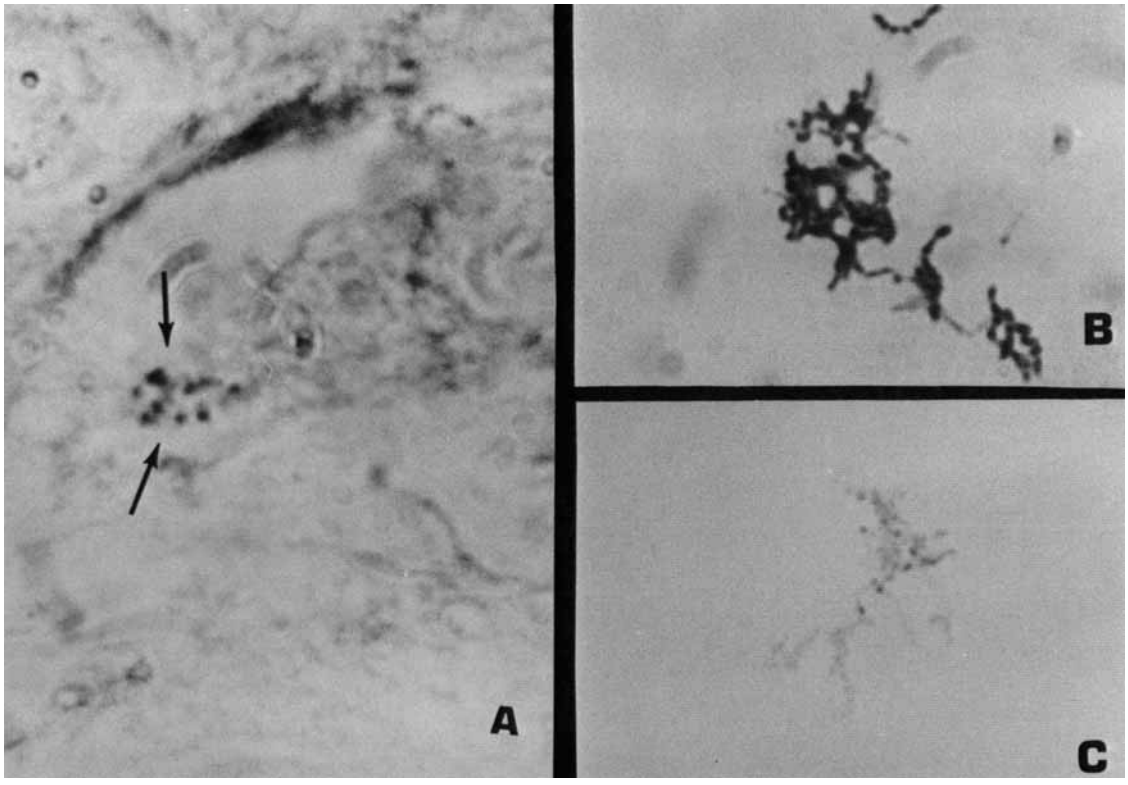

FIG. 4A (*left*). Generalized scleroderma (Case 3). Rare focus (*arrows*) of weakly acid-fast (pink) coccoid forms in the deep dermis. (Intensified Kinyoun stain, original magnification × 1000, in oil.) 4B (*top, right*) smear of Gram-positive coccobacilli isolated from skin biopsy material from the same case. Compare the size of the coccal elements to the in vivo coccoid forms observed in Fig. 4A. (Gram's stain, original magnification × 1000, in oil.) 4C (*bottom, right*) acid-fast stained smear of same culture illustrated in Fig. 4B. Compare the size of the non-acid-fast "granules" with those coccoid forms observed in Fig. 4A. (Ziehl-Neelsen stain, original magnification × 1000, in oil.)

Microscopic sections were stained for acid-fast bacteria by use of the Ziehl-Neelsen stain, the Fite stain, the Fite-Faraco stain, the Kinyoun stain, and the intensified Kinyoun stain for the detection of acid-fast cell-wall deficient forms of mycobacteria, as recommended by Mattman.[26] The slides were studied under oil-immersion magnification (×1000) with bright illumination.

The morphologic appearance, physiology, and pathogenicity of cell-wall deficient bacteria have been described in clinical proceedings edited by Guze[27] and in the monograph by Mattman.[28] The histologic appearance of purported cell-wall deficient bacteria in scleroderma,[9–11] hypodermitis sclerodermiformis,[18] and scleredema[19] has been reported elsewhere by Cantwell et al.

Results

Variably acid-fast coccoid forms were observed within the microscopic sections of all 20 patients. The histologic appearance of the coccoid forms was the same in all three diseases.

Figure 1 (Case 1) shows the typical appearance of two foci of purple-stained coccoid forms within the collagen bundles of the deep dermis in generalized scleroderma, as noted at high-power magnification (×400). Greater details of the forms can be observed with oil-immersion magnification (×1000) (Figs. 2A and B). These coccoid forms are approximately the size of brown or golden-stained melanin granules. Coccoid forms may be noted around the adnexae (hair shafts, pilosebaceous units, eccrine glands), as well as around the nerves and blood vessels (Fig. 3, Case 2). By use of the intensified Kinyoun stain, the coccoid forms could occasionally be stained weakly acid-fast (pink) (Fig. 4A, Case 3). These minute coccoid forms seen in Case 3 were similar to the in vitro granular coccoid elements of the coccobacillary, corynebacteria-like ("diphtheroid") microbes, which were cultured from the skin biopsy material from the same case (Figs. 4B and C, Case 3). Similar coccoid

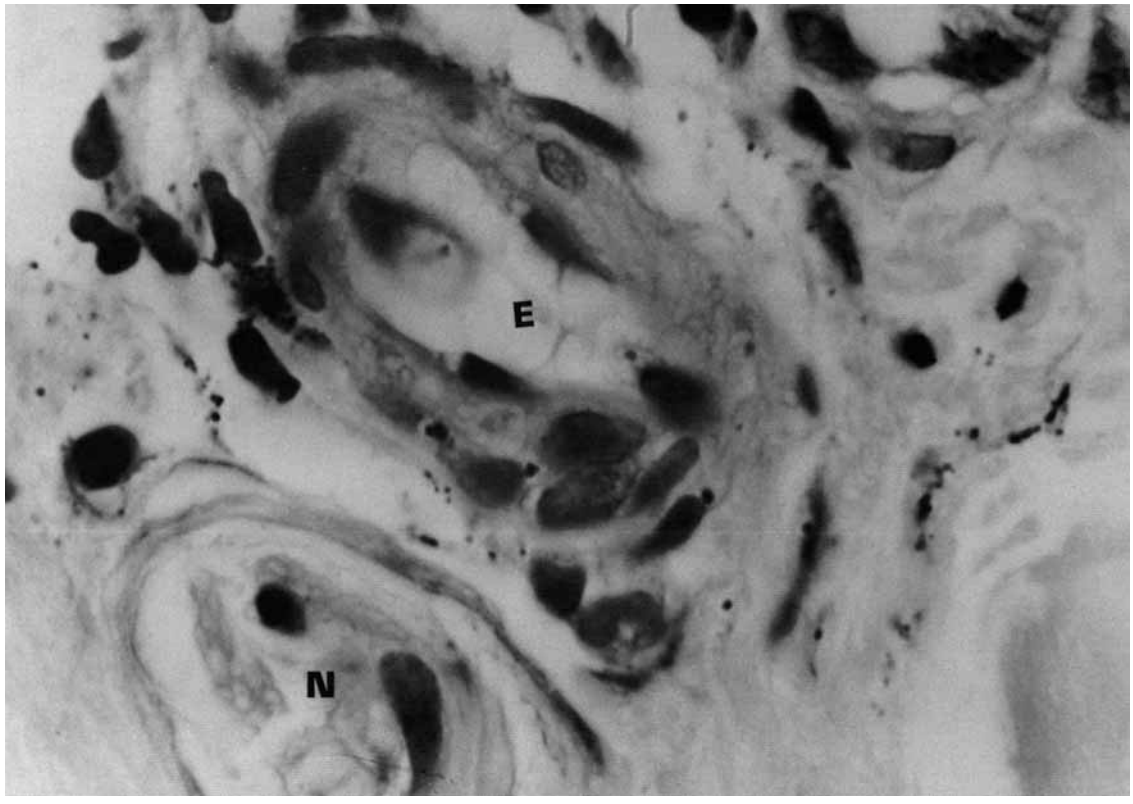

FIG. 5. Morphea. Numerous, scattered, purple-stained coccoid forms around an eccrine gland (E) and nerve (N) in the deep dermis. (Case 4; Ziehl-Neelsen stain, original magnification × 1000, in oil.)

forms could be observed around the eccrine glands and nerves (Fig. 5, Case 4), the blood vessels (Fig. 6, Case 5), and within the collagen bundles (Fig. *7A*, Case 6) in sections of morphea. These in vivo coccoid forms in Case 6 also resembled the pleomorphic cocci, which were isolated in culture from skin biopsy material from the same case (Fig. *7B*, Case 6).

Coccoid forms were observed within the dermis in sections of LSA. Figures *8A* and *B* illustrate coccoid forms, which were seen within the upper and mid-

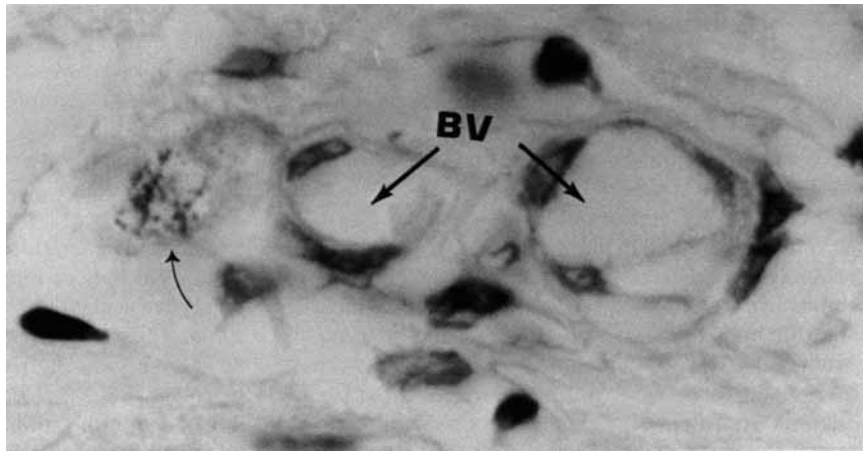

FIG. 6. Morphea. Non-acid-fast, purple-stained coccoid forms (curved arrow) adjacent to two deep dermal blood vessels (BV). (Case 5; Intensified Kinyoun stain, original magnification × 1000, in oil.)

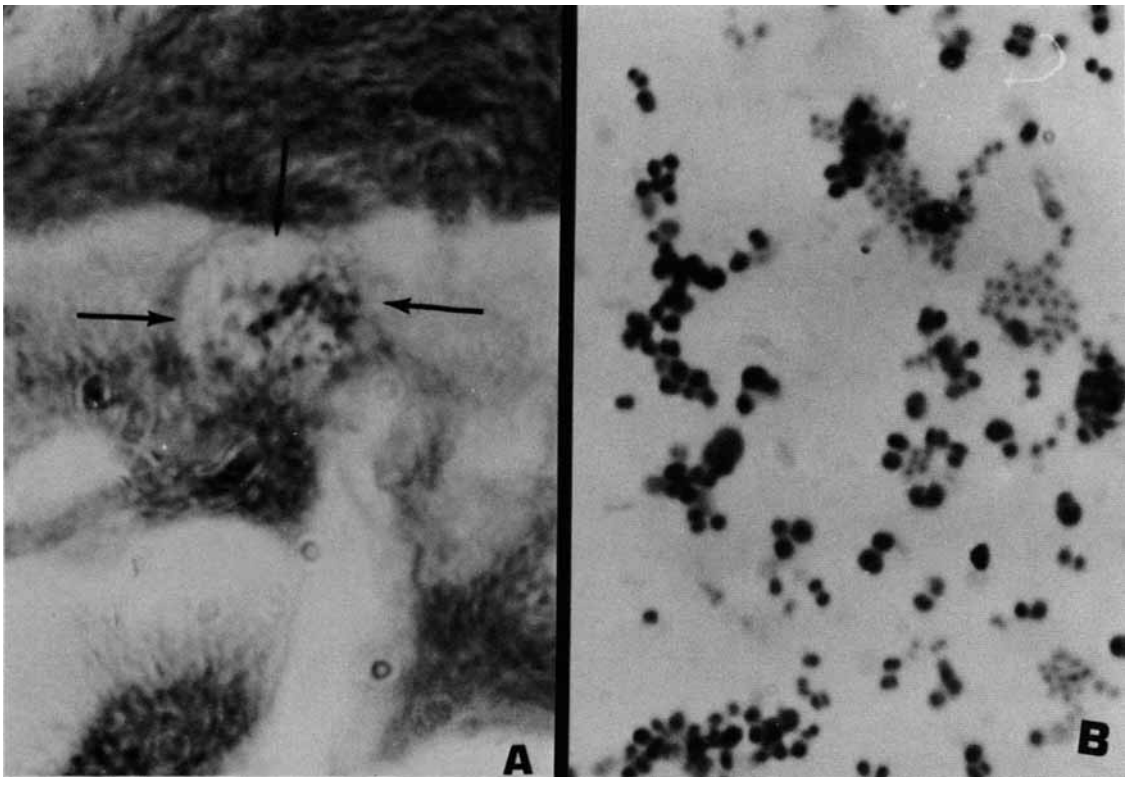

FIG. 7A (*left*). Linear morphea (Case 6). Rare focus (*arrows*) of non-acid-fast coccoid forms in a lacunar-like space in the collagen of the deep dermis. (Fite-Faraco stain, original magnification × 1000, in oil.) 7B (*right*) smear of variably sized, non-acid-fast, pleomorphic cocci isolated from this case. Compare the appearance of these cocci with the variously sized coccoid forms observed in vivo in Fig. 7A. (Ziehl-Neelsen stain, original magnification × 1000, in oil.)

dermis in areas showing the characteristic histopathologic changes of LSA, such as lymphedema of the upper part of the dermis with telangiectases, and a sparse inflammatory cell infiltrate.

Discussion

In my experience, variably acid-fast coccoid forms can always be identified in microscopic sections of scleroderma, morphea, and LSA. These forms may be stained weakly acid-fast by use of the intensified Kinyoun stain[26] designed for the demonstration of acid-fast, cell-wall deficient forms. The coccoid forms are ordinarily stained non-acid-fast (purple or blue) by use of routine acid-fast staining procedures. By use of the Fite stain, the microbes may stain purple (suggesting metachromasia) in contrast to the blue background tissue counterstain (Figs. 1–3, 8). Interestingly, certain cell-wall deficient forms of cocci and coccobacilli found in the hematologic elements,[29] as well as the "granules" of corynebacteria,[30] may stain metachromatically. Acid-fast rods are usually impossible to demonstrate in vivo in scleroderma and morphea, but occasionally they have been observed in smears and microscopic sections.[1,5–8] Foci of clumped or scattered coccoid forms are most often found in the deeper portions of the dermis (Figs. 1, 2, 4A, and 7A), and around the adnexal structures (Figs. 3 and 5). Less commonly, microbes are found around the dermal blood vessels (Fig. 6). Coccoid forms were never observed within the epidermis. Ordinarily, coccoid forms are not visible in hematoxylin-eosin stained sections.

Intra-eccrine acid-fast "granules," "bodies," or coccoid forms may or may not be observed within the eccrine sweat glands. Cantwell et al has suggested that some of these acid-fast forms might represent cell-wall deficient bacteria carried over from the blood stream.[9,10] However, Rahbari[31] believes these acid-fast bodies are normal, non-microbial lipofuscin pigment. The histologic difficulties encountered in differentiating lipofus-

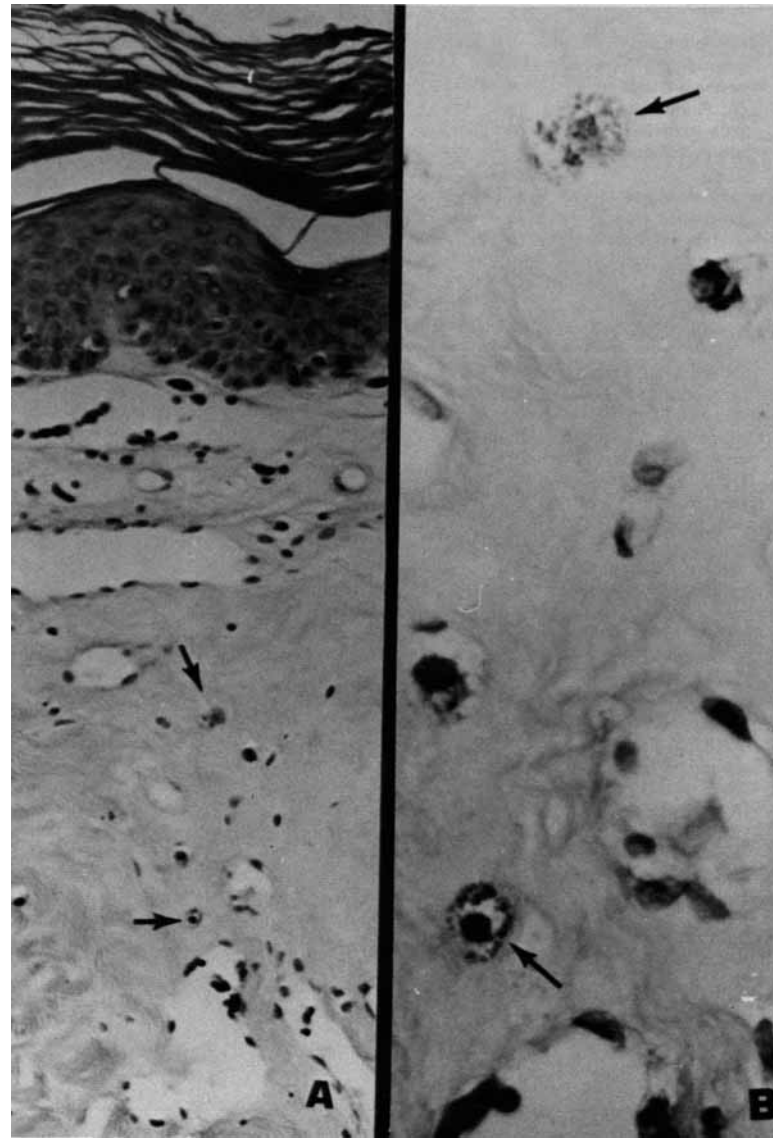

FIG. 8A (*left*). Lichen sclerosus et atrophicus (Case 7). Section showing characteristic lymphedema of the upper and mid-dermis with telangiectases and a sparse inflammatory infiltrate. Arrows point to foci of coccoid forms (Fite stain, original magnification × 100). 8B (*right*) same section as in Fig. 8A showing the same two foci of non-acid-fast purple-stained coccoid forms (*arrows*) at higher magnification. Note the lacunar-like space around the forms. (Fite stain, original magnification × 400.)

cin pigment from L forms of bacteria has been discussed in great length by Moscovic[32] in his thesis concerning the role of L forms in sarcoidosis.

The precise role of cell-wall deficient bacteria (L forms) in human disease is not known. However, there is increasing evidence to suggest that the hematologic elements of all human beings are infected with cell-wall deficient microbes.[29,33] Some of these microbes may be acid-fast.[29] In view of these findings, some consideration should be given these microbes as possible pathogenic agents, especially in diseases of unknown etiology.

Variably acid-fast coccoid forms in scleroderma, morphea, and LSA must be differentiated from melanin pigment granules and hemosiderin pigment granules. These forms stain brown with acid-fast staining procedures. Mast cells with their "granules" can be differentiated by the presence of a mast cell nucleus. There is no evidence to suggest that the acid-fast forms in this report are "artifacts" or "contaminating microbes."

It is likely that the hypothesis implicating an acid-fast microbial agent in scleroderma, morphea, and LSA will be strengthened only by the consistent in vivo demonstration of such microbes in microscopic sections showing the characteristic histopathologic changes. Further histologic studies by other investigators are sorely needed to prove or disprove this hypothesis.

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Toxic Oil Syndrome

There is almost conclusive epidemiological evidence that an epidemic disease in Spain resulted from ingestion of food oil purchased from itinerant vendors; the disease is now officially called "toxic oil syndrome." The epidemic is over but during its course some 20,000 people were affected with over 350 deaths between early May, 1981, and the end of 1982. The most commonly reported symptoms were fever, respiratory distress, nausea and vomiting, various skin eruptions, general discomfort, headaches, abdominal pain, and myalgias. The radiological picture was consistent with intense pleuropulmonary transudation and eosinophilia. This clinical picture with negative microbiological studies represent a new disease. Some patients at this stage died from respiratory failure or thrombosis of the mesenteric arteries and veins or pulmonary thromboembolism. The chronic phase is characterised by neuromuscular changes ranging from simple myalgia to muscular weakness and atrophy involving peripheral neuropathy, severe weight loss, scleroderma-like skin changes, and pulmonary hypertension. The most common finding in the disease is endothelial injury, which may account for many of the other sequelae. Whether the disease picture is still developing is not clear.— *Toxic oil syndrome. Lancet i:1257, 1983*