Acta Dermatovener (Stockholm) 50: 119–124, 1970

AQUARIUM-BORNE INFECTION WITH MYCOBACTERIUM MARINUM

Tore Månsson, Eva Brehmer-Andersson, Barbro Wittbeck and Rune Grubb

From the Departments of Dermatology, Pathology and Medical Microbiology, University of Lund, Lund, Sweden

Abstract. Mycobacterium marinum was isolated from a lesion on the lower arm in a man, aged 55. A typical tuberculoid inflammation did not develop until $5\,^1/_2$ months after the onset. The bacteria could be traced to the patient's aquarium, where they were cultured from sand, snails and fish of the species Gymnocorymbus ternetzi that died from a disease with granulomas in their fins and liver. The infection had been introduced into the aquarium with water fleas used for feeding the fish. The water fleas had been caught in a pond and also from its mud M. marinum was cultured.

Infections with Mycobacterium marinum generally occur epidemically among persons who have visited an infected swimming-pool. Large epidemics have been reported from Scandinavia, USA and England (8, 10, 18, 20). Sporadic cases of infection with M. marinum are also on record. In three of these cases the source of infection was an aquarium (9, 16). A case of infection with M. marinum transmitted from a pond via an aquarium is described below.

CASE REPORT

The patient was a 55-year-old male factory worker.

Heredity. The patient was the fourth of eight siblings. One brother had died at 49 years from pulmonary sarcoidosis which he had had for 11 years. One sister had had symptoms of pulmonary sarcoidosis.

Previous diseases. In 1966 the patient had had acute bronchitis. The intracutaneous tuberculin test (Old tuberculin) 1 mg had been positive (indurated area 10 mm in diameter) and chest X-ray had shown moderate emphysema and signs of previous pleurisy.

Present disease. At the end of September 1967 an initially rapidly growing infiltrate developed on the dorsal aspect of the right lower arm without any known preceding trauma. The patient had no itching or other symptoms. When first seen at the Department of Dermatology 3 weeks later he had a 4×2.5 cm bluish-red exfoliative lesion with marginal pustules. Though no mycelia could be demonstrated, mycosis was suspected, and antimycotic treatment was started.

Antimycotic therapy for 6 weeks had no demonstrable effect. Biopsy showed non-specific inflammation with epithelial hyperplasia. The intracutaneous tuberculin test (Old tuberculin) 1 mg was strongly positive (induration 25–30 mm in diameter). Chest X-ray showed no parenchymal changes and no enlargement of mediastinal lymph nodes. Neither could any enlarged lymph nodes be palpated. Culture of material from the pustules gave no growth of pyogenic bacteria. Tubercle bacilli could not be demonstrated in direct smears. The blood picture was normal, E.S.R. was 2 mm/1 hour and serological tests for syphilis were negative.

The appearance of the lesion 4 months after the onset is illustrated in Fig. 1. The blue-red area was covered centrally by crusts, under which were numerous pustules and exuberant granulation tissue.

Renewed biopsy suggested the possibility of pyoderma gangrenosum. The patient was treated locally with fluocinolone acetonide, during which therapy pustulation increased. Following oral treatment with steroids (prednisolone 60–40 mg daily) the lesion became less infiltrated and tended to heal centrally. However, culture for acid-fast bacteria of pus from the lesion gave growth of Mycobacterium marinum. Steroid treatment was therefore stopped.

No treatment has been given since April 1968, by which time the disease had persisted for 6 months. The lesion successively healed in the centre with serpiginating progress at the periphery. Fig. 2 shows the appearance of the lesion 13 months after the onset and 9 months after the stage illustrated in Fig. 1.

Histological examinations. Punch biopsy was done on 4 occasions: Dec. 5, 1967, Jan. 16, March 19, and April 10, 1968. Sections were stained with hematoxylin-eosin and according to van Gieson, Ziehl-Neelsen and Hotchkiss–McManus (PAS). Later on the rests of the specimens were serially cut and stained according to Ziehl-Neelsen, but with a shorter decoloration time, 1 min against the usual 5 min, in hydrochloric alcohol (1% HCl in 70% alcohol).

Biopsy 1, Dec. 5, 1967: the picture was unspecific with irregular epithelial hyperplasia and a dense infiltrate of lymphocytes, plasma cells, swollen histiocytes and polynuclear leucocytes deep in the dermis.

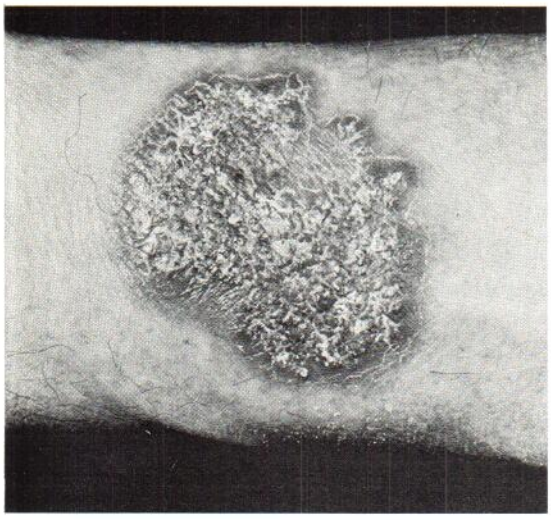

Fig. 1. Appearance of lesion four months after onset.

Biopsy 2, Jan. 16, 1968, 15 weeks after onset: the epithelial hyperplasia was considerable and appeared to affect mainly the hair follicles. Abscesses were seen in hair follicles, in the epidermis and in the dermis. The dermis also contained a dense unspecific, inflammatory cell infiltrate as well as scattered Langhans's giant cells and two small epithelioid cell granulomas.

Biopsy 3, March 19, 1968, obtained about 6 months after the onset of the disease: distinct epithelioid cell granulomas of varying size had now developed in the upper and middle part of the corium.

Punch biopsies April 10, 1968: the epithelioid cell granulomatosis was more marked than at the previous examination. The epithelioid cell foci contained both Langhans's giant cells and foreign body giant cells, but no necroses were found. Some granulomas were arranged round severely changed hair follicles. Fig. 3 shows a wide, sacculated follicle. The duct is plugged by a dense mass of disintegrating polymorphonuclear leucocytes and in the corium the follicle is surrounded by epithelioid cell granulomas. Some areas in the dermis contained very dense, chronic inflammatory cell infiltrates as well as small, dense accumulations of polymorphonuclear leucocytes.

No spores, hyphae or acid-fast bacilli could be demonstrated in any of the specimens.

Epidemiological investigations. The patient had not visited a swimming-pool for many years. But he had an aquarium with some thirty sorts of fish and snails of the species Melanoides tuberculata.

In view of previous reports in the literature of human infection with M. marinum from aquarium snails (9), apparently healthy snails and sand and water from the patient's aquarium were cultured for M. marinum.

In the autumn of 1967 the patient had fed his fish with water fleas, Daphniae, which he had caught in two different field ponds. It is well-known among aquarium owners that the mortality rate among fish in aquaria increases every autumn. This is supposed to result from feeding with water fleas. In the autumn of 1967 many of the fish in the patient's aquarium had died from a disease with ulcerations of the fins. In the spring and summer of 1968 the fish were in good condition. In September the patient again began to feed the fish with water fleas from the same two ponds as before. After a few weeks two fishes of the species Gymnocorymbus ternetzi died. A tumour, the size of a pea, was found on the tail of one of these fishes (Fig. 4). The tumour and the liver from this fish were examined histologically and material was taken from both fishes for culture for mycobacteria. Water fleas and mud from the two ponds were also taken for culture for M. marinum.

Histological examination of G. ternetzi (Fig. 5). The tumour and liver tissue were examined. The tumour consisted of crowded, well outlined, round granulomas. The granulomas were built up of macrophages with eosinophilic ill-defined cytoplasm and relatively large, rounded,

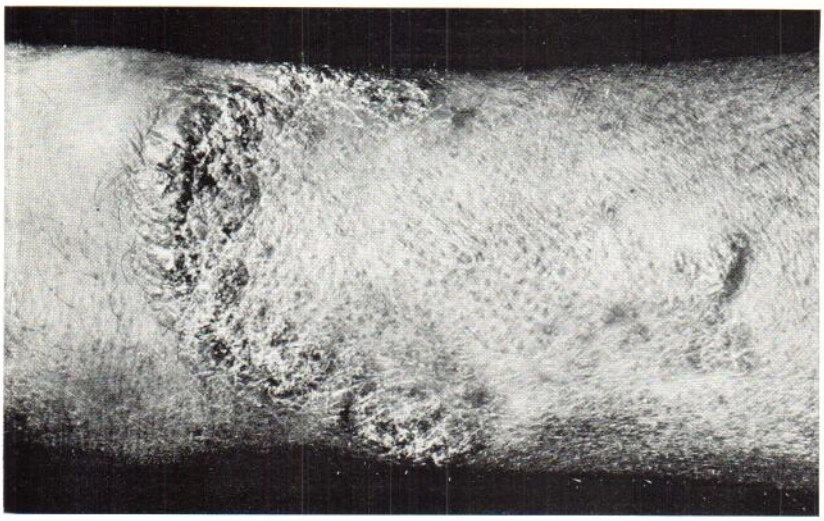

Fig. 2. Appearance of lesion thirteen months after onset.

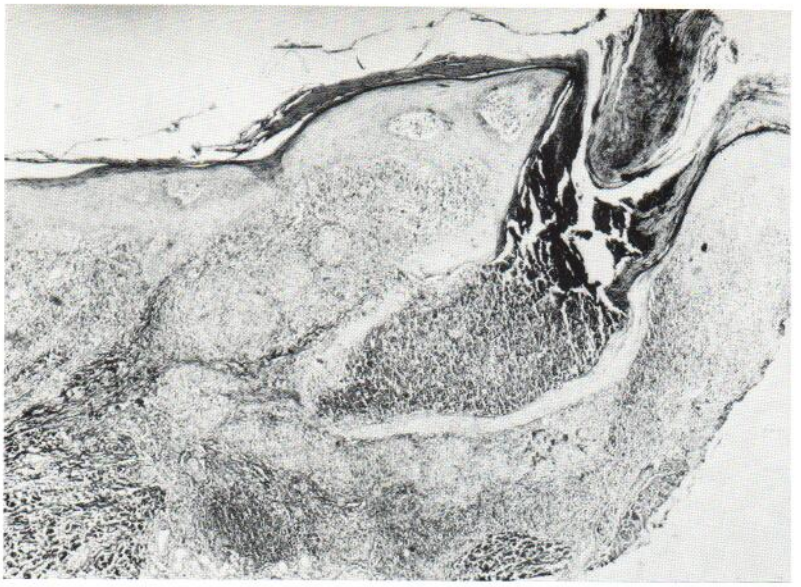

Fig. 3. Sacculated hair follicle, densely filled with polynuclear leucocytes. In the dermis the follicle is surrounded by epithelioid cell foci. Van Gieson, × 42.

loose nuclei. No giant cells were seen. Narrow rings of fibroblast-like cells surrounded the granulomas, most of which showed central necroses. Staining according to the modified Ziehl-Neelsen technique revealed numerous acid-fast bacilli in the granulomas, mainly in the necrotic foci. The rods were long and slender and some showed a beaded appearance. The liver tissue contained scattered granulomas of the same appearance as in the tumor.

Bacteriological studies. Cultures for M. marinum were made on Löwenstein–Jensen's medium at 31°C according to the method of Linell & Nordén (8). The diagnostic criteria used were:

1. Acid-fast rods with optimal growth at 31°C and little or no growth at 37°C.
2. Photochromogenicity together with colony appearance.
3. Lack of pathogenicity for guinea-pigs.
4. Typical lesions, especially at the tail of male C_3H mice, injected intraperitoneally with the culture as described by Linell & Nordén. Such lesions are demonstrated in Fig. 6.

Table I (p. 122) gives a list of the sites from which bacteria with the above-mentioned characteristics were cultured.

M. marinum was thus demonstrated not only in the patient's lesion but also in sand, snails and infected Gymnocorymbi from his aquarium. Bacteria were also recovered from one of the ponds where he caught water fleas for his fish. Culture of water from his aquarium and from another pond providing water fleas, gave no growth of M. marinum.

Studies of the susceptibility of the bacteria to various drugs showed resistance to paraaminosalicylic acid, isoniazid, streptomycin, kanamycin, viomycin, cycloserine, thiacetazone, pyrazinamide, tiocarlide and 15 other commonly available antibiotics or antibacterial drugs. Erythromycin was inhibitory to some extent, its minimum inhibitory concentration being 12.5 μg/ml.

DISCUSSION

M. marinum was originally identified in 1926 as the cause of spontaneous tuberculosis in salt-water fish (1). Later experimental investigations (3) have shown it to be pathogenetic also for fresh-water fish, snakes, frogs and tortoises. Infections with this or a very closely related mycobacterium (M. platypoecilus) have been demonstrated in the Mexican platyfish (Platypoecilus maculatus),

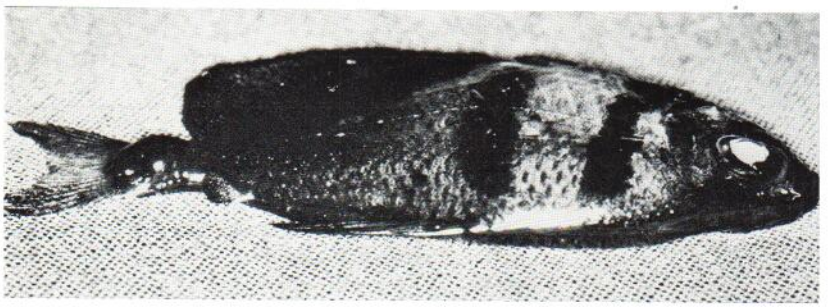

Fig. 4. Gymnocorymbus ternetzi with a granuloma from Mycobacterium marinum on the tail.

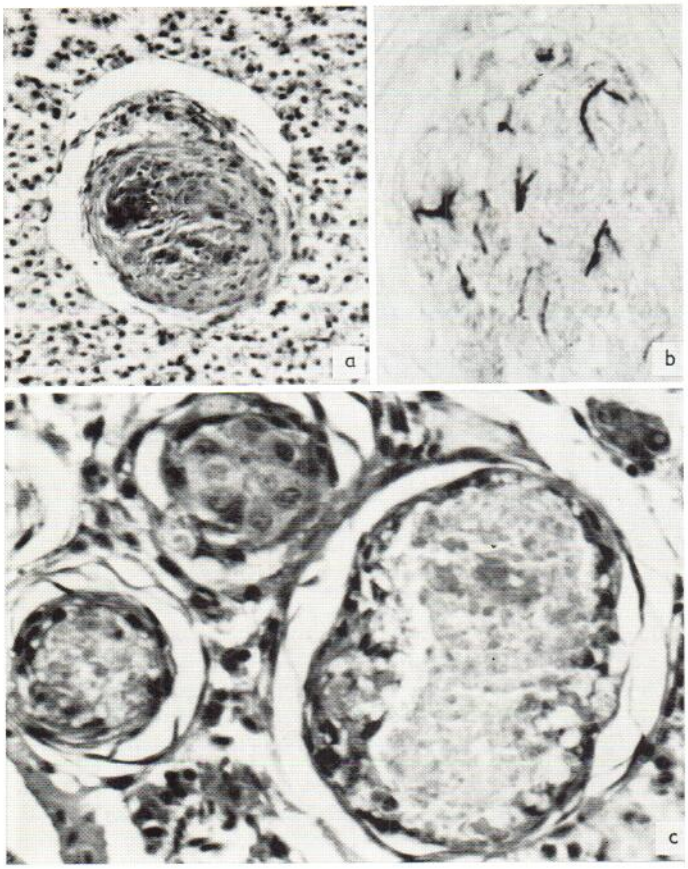

Fig. 5. (*a*) Granuloma in the liver of Gymnocorymbus ternetzi. Haematoxylin-eosin, × 210.
(*b*) Mycobacterium marinum in granuloma of the liver. Some bacteria have a beaded appearance. Ziehl-Neelsen, × 1040.
(*c*) Three granulomas outlined by fibroblast-like cells. The two lower granulomas are extensively necrotic. The upper one is built up by well perserved macrophages. Haematoxylin-eosin, × 330.

causing inter alia ulcerations in the region of the dorsal fins (2), i.e. lesions resembling those our patient had observed in his aquarium fish. Nodular lesions similar to those observed in one of the Gymnocorymbi in this investigation have earlier been observed in infection of aquarian fish with M. marinum (15). The histological changes in the tumour and liver of the G. ternetzi in our case are compatible with earlier descriptions of tuberculosis in fish (1, 2, 12).

In 1954 Linell & Nordén demonstrated that an epidemic of tuberculoid skin lesions in a Swedish town was caused by an acid-fast rod, for which they coined the name Mycobacterium balnei (8). Later investigations have shown that M. marinum and M. balnei are identical (3).

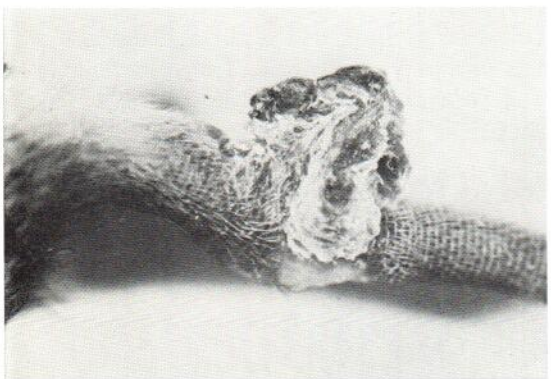

Fig. 6. Ulcerations on the tail of a C_3H male mouse inoculated with Mycobacterium marinum intraperitoneally.

Table I. *Lesions and sites from which Mycobacterium marinum has been cultured*

Date	
24.1.68	Pus from patient's lesion
20.3.68	Punch-biopsy specimen from the lesion
11.4.68	Snails (Melanoides tuberculata) from aquarium
3.8.68	Sand from aquarium
25.10.68	Tumour tissue and tail lesion from Gymnocorymbus ternetzi
26.10.68	Water fleas and mud from Habo pond

The greatest number of human infections with M. marinum have occurred as epidemics, whose origin has been traced to infected swimming-pools (8, 10, 11, 18, 20). Also most sporadic cases have apparently originated from swimming-baths (e.g. 4, 17). Granulomas after contact with infected aquaria have also been reported. In one case infected snails were thought to be the source of infection (9). In two others it was found that both persons had been in contact with a fish tank infected with M. marinum (16).

The finding of bacteria in snails and fish in our patient's aquarium suggests that he had been infected from this source. The demonstration of M. marinum in water fleas and mud from one of the ponds clearly indicates the route of infection to the aquarium. That the occurrence in nature of these bacteria is not confined to swimming-pools, has been demonstrated also in previous studies. Thus, a girl in Linell & Nordén's series had been infected in a lake (8) and another girl in the water of the river Potomac (5).

In human beings M. marinum can produce lesions of varying appearance. In Linell & Nordén's series the infiltrate usually ulcerated (8), while in that of Philpott et al. the majority of the lesions remained intact without ulceration or necrosis (10). The latter series included some cases with ascending multiple limb-lesions resembling sporotrichosis. In our case central healing of the lesion was accompanied by marked serpiginous marginal inflammatory activity. This picture resembles most that described in Orientals (6, 14, 19).

The occurrence of sarcoidosis in two of the patient's siblings is noteworthy. In one of the above-mentioned Orientals infected with M. marinum X-ray examination revealed small cystic rarefactions in the metacarpals and phalanges of the fingers on both sides. The picture was considered compatible with sarcoidosis (6). A patient with coexisting pulmonary sarcoidosis and infection with M. marinum of the skin has recently been observed (7).

Histological examination of 15 of the lesions of varying ages in the epidemic of swimming-pool granulomas in Colorado, USA in 1959 showed that both early and late in the course the microscopic picture can be non-specific (13). Only in one-third of the lesions was the inflammation tuberculoid. In the early stage of our case, as in many other cases on record, the histological picture was dominated by epithelial hyperplasia and a dense infiltrate of chronic inflammatory cells in the corium. In addition, abscesses were seen, above all in and around the hair follicles. Dense infiltrates of polymorphonuclear leucocytes were seen only in one of the cases in the Colorado-epidemic and in none of the 31 biopsy specimens in Linell & Nordén's series (8).

In an epidemic in Penarth in South Wales 1966–67 biopsies were obtained from 12 out of 81 patients (18). The histological findings varied with the age of the lesion. No tuberculoid granulomas were demonstrated in biopsy specimens obtained within two months of the onset of the lesion.

Histological studies have been made on lesions of varying age also in previous reports, but each patient was examined on one occasion only. Our case was followed up with repeated biopsy. True epithelioid cell granulomas were not observed until $5\,^1/_2$ months after the onset of the disease. After $3\,^1/_2$ months scattered Langhans's giant cells and two small epithelioid cell foci were seen, interpreted then as secondary to the very pronounced folliculitis. They were, however, presumably incipient epithelioid cell granulomas.

In Linell & Nordén's series acid-fast rods were found in histological sections in one out of 31 cases, in the Colorado-epidemic in 9 out of 15 and in the South Wales epidemic in 1 out of 12 cases. In none of the 5 biopsy specimens from our patient were acid-fast bacilli observed.

Since the bacteria cultured from our patient were resistant to the tuberculostatic drugs studied, no specific therapy was tried. The clinical response of swimming-pool granulomas to tuberculostatics may sometimes be better than that suggested by bacteriological susceptibility tests (10). These compounds should, however, be used with caution because of their side-effects. The disease is benign and most infections heal spontaneously, though often only slowly. Several lesions have existed for more than 4 years, and one persistent for 45 years has been described (6, 14, 19).

Our case illustrates how difficult it is to make a clinical diagnosis of sporadic infections with M. marinum. The non-specific histological picture in some stages of the disease makes the diagnosis still more difficult. The possibility of an aquarium-borne infection should be considered in sporadic cases of infection with M. marinum. In

our case a hitherto unknown way from a natural reservoir of the bacteria to human infection has been demonstrated.

REFERENCES

1. Aronson, J.: Spontaneous tuberculosis in salt-water fish. J infect Dis *39*: 315, 1926.
2. Baker, J. & Hagan, W.: Tuberculosis of the Mexican platyfish (Platypoecilus maculatus). J infect Dis *70*: 248, 1942.
3. Clark, F. & Shepard, C.: Effect of environmental temperatures on infection with Mycobacterium marinum (balnei) of mice and a number of poikilothermic species. J Bact *86*: 1057, 1963.
4. Cleveland, D.: Possible tuberculous skin infection from a swimming pool: Report of 4 cases. Acta Dermatovener (Stockholm) *31:* 147, 1951.
5. Cott, R., Carter, M. & Sall, T.: Cutaneous disease caused by atypical mycobacteria: Report of two chromogen infections and review of the subject. Arch Derm (Chicago) *95:* 259, 1967.
6. Gould, W., McMeekin, D. & Bright, R.: Mycobacterium marinum (balnei) infection: Report of a case with cutaneous and laryngeal lesions. Arch Derm (Chicago) *97:* 159, 1968.
7. Lindeskog, G.: Personal communication.
8. Linell, F. & Nordén, Å.: Mycobacterium balnei: A new acid-fast bacillus occurring in swimming pools and capable of producing skin lesions in humans. Acta Tuberc Scand, Suppl., *33:* 1, 1954.
9. Pegum, J. S.: Clinical features of some atypical mycobacterial infections. Trans St John's Hosp Derm Soc *53:* 119, 1967.
10. Philpott, J., Woodburne, A., Philpott, O., Schaefer, W. & Mollohan, C.: Swimming pool granuloma: A study of 290 cases. Arch Derm (Chicago) *88:* 158, 1963.
11. Rees, R. & Bennett, J.: Granuloma following swimming pool abrasion. JAMA *152:* 1606, 1953.
12. Reichenbach-Klinke, H.-H.: Krankheiten und Schädigungen der Fische, pp. 58–62. Stuttgart, 1966.
13. Schaefer, W. & Davis, C.: A bacteriologic and histopathologic study of skin granuloma due to Mycobacterium balnei. Amer Rev Resp Dis *84*: 837, 1961.
14. Sommer, A., Williams, R. & Mandel, A.: Mycobacterium balnei infection: Report of two cases. Arch Derm (Chicago) *86:* 316, 1962.
15. Sterba, G.: Aquarienkunde. Aquarien-Technik, Biologie, Ökologie und Anatomie der Fische, Einzelbeschreibung der Arten, Band 2, p. 114. Berchtesgaden, 1955–56.
16. Swift, S. & Cohen, H.: Granulomas of the skin due to Mycobacterium balnei after abrasions from a fish tank. New Engl J Med *267:* 1244, 1962.
17. Tolmach, J. & Frank, S.: Granuloma of skin with tubercle formation following swimming pool injury. JAMA *151*: 724, 1953.
18. Waddingtong, E.: An outbreak of swimming pool granuloma. Trans St John's Hosp Derm Soc *53*: 122, 1967.
19. Walker, H., Shinn, M., Higaki, M. & Ogata, J.: Some characteristics of "swimming pool" disease in Hawaii. Hawaii Med J *21*: 403, 1962.
20. Zettergren, L., Zetterberg, B., Malmgren, B. & Karth, B.: Nya rön beträffande simhallssjukdomen (mycobacteriosis balnearea). Svensk Läkartidn *49*: 2936, 1952.

Received August 26, 1969

Tore Månsson, M.D.
Department of Dermatology
University of Lund
Lund
Sweden