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Serological Studies of *Actinomyces israelii* by Crossed Immunoelectrophoresis: Taxonomic and Diagnostic Applications

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Crossed immunoelectrophoresis (CIE) with intermediate gel was applied to the serological analysis of *Actinomyces israelii* to develop a test with high efficiency in the laboratory diagnosis of human actinomycosis and classification of *A. israelii*. Recently developed standard antigen-antibody systems for *A. israelii* by CIE were used as reference. The reference systems were based on standard preparations of cytoplasmic and whole cell-associated antigens of *A. israelii* and a standard immunoglobulin G pool purified from rabbit antisera to formalin-treated whole cells and cell lysates of *A. israelii*. The specificity of the standard antigens for *A. israelii* was evaluated in CIE studies by screening for antibodies to components of the antigens in rabbit antisera raised against related bacteria. The standard system for *A. israelii* based on cytoplasmic antigens formed species-specific precipitins, whereas antisera raised against *A. naeslundii* and/or *Propionibacterium acnes* precipitated components of the other standard antigens. As a result of these analyses, the standard system for *A. israelii* based on 10 cytoplasmic antigens was used as reference for CIE studies to detect humoral antibodies to *A. israelii* in sera from nine patients with actinomycosis. All the sera from the patients formed at the time of diagnosis one or more precipitins in terms of the 10 reference precipitins. Up to five precipitins were found in single sera. Follow-up studies covering a period of one-half year after treatment showed a gradually decreased precipitin response in the course of time. In control sera from patients with newly diagnosed tuberculosis, nocardiosis, deep *Candida* infection, and aspergillosis, and in sera from healthy blood donors, no antibodies were detected with specificity for the reference antigens.

Actinomyces israelii is the most common etiological agent of actinomycosis in man (9, 14, 21), although other species of the genus *Actinomyces* and *Arachnia propionica* have also been implicated (7, 10). Actinomycosis is probably often missed in humans, since the laboratory diagnosis of this disease depends on the demonstration of *A. israelii* in exudates and/or the isolation of this organism in culture (9). Diagnostic problems include the requirement of special arrangements for sample taking and for culture conditions. In many cases antibiotic therapy is started before the true identity of the causative organism is established. A reliable serological test would therefore be a useful adjunct in the diagnosis and prediction of the prognosis of the disease. Moreover, it may often be the only criterion of current or previous actinomycosis.

Antisera raised in rabbits against strains of *A. israelii* have in immunofluorescence, precipitin tests, and cell wall agglutination tests allowed differentiation of a distinct serogroup of *A. israelii*, as well as two serotypes (4, 6, 7, 8, 20, 24).

Even though considerable work has been done on the production of specific antisera to identify and classify *A. israelii*, conflicting results are to be found in the literature concerning serum titers against *A. israelii* in patients with proved actinomycotic infections and concerning cross-reacting antibodies against antigens of *A. israelii* formed in other diseases (11, 15). However, little basic work has been done on the characterization of antigen preparations of *A. israelii* and the development of generally acceptable techniques for the conduct of serological tests to diagnose actinomycosis. The immunodiffusion tests, hemagglutination tests, and complement fixation tests have been the basic serological methods for studies on the serological diagnosis of actinomycosis.

Crossed immunoelectrophoresis (CIE) with intermediate gel (1) proved to be a powerful method in combination with a reference precipitin system for characterization of antibodies in mucocutaneous candidiasis (3), *Pseudomonas aeruginosa* infections (18), and *Mycobacterium leprae* infections (2). The objectives of this study were to apply CIE with intermediate gel technique to the serological diagnosis of human actinomycosis and classification of *A. israelii*, using recently developed standard antigen-antibody systems as reference (17).

MATERIALS AND METHODS

Strains. For immunization of rabbits the following type strains were used: *A. israelii*, serotype 1, ATCC 12103, SBL 225/74; *A. israelii*, serotype 2, ATCC 23036, WVU 307; *A. viscosus*, serotype 1, ATCC 15987; *A. viscosus*, serotype 2, ATCC 19246; *A. naeslundii*, ATCC 12104; *A. odontolyticus*, serotype 1, ATCC 17982; *A. odontolyticus*, serotype 2, WVU 482; *Arachnia propionica*, serotype 1, ATCC 14157; *A. propionica*, serotype 2, WVU 346; *P. acnes*, serotype 1, NCTC 737; *P. acnes*, serotype 2, ATCC 11828; *P. avidum*, ATCC 25277; *P. granulosum*, ATCC 25264; *Nocardia asteroides*, ATCC 19247, obtained from American Type Culture Collection (ATCC), West Virginia University (WVU), and National Collection of Type Cultures (NCTC).

Antisera. Rabbit antisera against *Actinomyces* spp., *A. propionica*, *Propionibacterium* spp., and *N. asteroides* were prepared by intravenous injections of formalin-killed whole cells by an immunization schedule previously described (16). Two antisera against each strain from different rabbits were pooled, and the immunoglobulin fraction was purified by salting out with ammonium sulfate at 37% saturation. The antibody content of each pool was concentrated to 10 mg/ml. Also included in the study were commercial rabbit antiserum to *Candida albicans* (obtained from Dakopatts A/S, Copenhagen, Denmark), rabbit antiserum to *Aspergillus fumigatus* (prepared in this institution), and rabbit antisera to *Aspergillus fumigatus*, *Thermoactinomyces vulgaris*, and *Micropolyspora faeni* (kindly supplied by D. W. R. MacKenzie, Mycological Reference Laboratory, Public Health Laboratory Service, Colindale, England).

Human sera were obtained from nine patients with actinomycosis diagnosed on the basis of clinical and laboratory findings. Five patients suffered from a cervicofacial form and four from thoracic actinomycosis. In each case the clinical diagnosis was obtained from the attending physician. The infecting organisms were identified as *A. israelii* by the criteria of K. Holmberg and C.-E. Nord (J. Gen. Microbiol., in press). Six patients were followed through remission with consecutive serum specimens. Serum specimens were collected at the time of diagnosis, at 2 months, at 4 months, at 6 months, and sometimes at 1 year after establishment of the diagnosis.

Serum specimens, pooled in batches of 10 from 50 adult healthy donors selected at random, and pooled serum specimens from 20 children aged 1 to 10 years were included as controls. Serum specimens from 10 newly diagnosed tuberculosis patients, two nocardiosis patients, two patients with deep candidosis, and three patients with pulmonary aspergilloma were tested for precipitating antibodies to *A. israelii*. The diagnosis of tuberculosis and nocardiosis were proven by isolation of *Mycobacterium tuberculosis* and *N. asteroides*, respectively. Candidosis was diagnosed on the basis of isolation of *C. albicans* and positive precipitation reaction in double immunodiffusion tests. Aspergillosis was proven by isolation of *A. fumigatus* and positive immunodiffusion tests. In addition, sera from two patients with chronic acnes vulgaris from which *P. acnes* was isolated and ten patients with chronic periodontal disease from which *A. israelii* was isolated (Holmberg, Arch. Oral Biol., in press) were included for tests. All sera were stored at −20 C with 15 mM NaN_3 as preservative.

Standard antigen-antibody systems for A. israelii. Reference antigen-antibody systems for *A. israelii*, were prepared from antigen/water extracts on standard preparations of sonic lysates, StAgSL, and chemical extracts from whole cells by 0.2 M hydrochloric acid (StAgHCl), 4 M urea (StAgurea), and 10% (vol/vol) trichloroacetic acid (StAgTCA), and standard rabbit antisera to formalin-treated whole cells (StAbI) and cell lysates (StAbII) of *A. israelii* (ATCC 12103 and WVU 307). The procedure for preparing standard antigens, standard antisera, and the immunochemical characteristics of the systems have been described elsewhere (17).

The standard system based on StAgSL and StAbI comprised six reference precipitins, coded SL1 to SL6, the system based on StAgHCl comprised five reference precipitins, coded HCl1 to HCl5, the system based on StAgurea comprised five reference precipitins, coded urea1 to urea5, and the system based on StAgTCA comprised one reference precipitin, coded TCA1. The reference precipitins coded SL1, SL2, SL4, SL5, HCl1, HCl2, HCl3, urea1, urea2, and urea-4 were specific for their respective systems, whereas the other reference precipitins of the systems showed reactions of partial or total identity to each other in CLE analysis (17). The system based on StAgSL and StAbII comprised 10 reference precipitates, coded 1 to 10, of which six were identical to those revealed in the system based on StAgSL and StAbI. None of the remaining precipitates was identical to any of the precipitates in the other systems employed.

CIE with intermediate gel. These immunoelectrophoreses were run by the procedures devised by Axelsen (1), using the same basic reagents as described previously (17). Antigen (5 μl) was submitted to the first-dimension electrophoretic run (9 to 10 V/cm) for 50 min. The second-dimension electrophoresis was run overnight, applying 2 V/cm. The reference gel contained 5 μl of the standard preparation of anti-*A. israelii* serum StAbII per cm^2. The intermediate gels contained 15 μl of the test serum per cm^2. A negative control plate, containing the same amount of saline instead of antiserum in the intermediate gel, and a positive control plate, containing the reference antiserum, were always run with each set of six electrophoreses. First- and second-dimension

electrophoreses and the pressing, washing, and staining of the plates were carried out as described previously (17). Analyses of the immunoprecipitation patterns were performed by comparison with the control plates. Differences in precipitin pattern between the test plates and the control plates were interpreted according to Axelsen (1).

RESULTS

By means of CIE with intermediate gel using the standard antigen-antibody systems for *A. israelii* as a reference, the serological relationship between *A. israelii* and taxonomically related bacteria was studied. Screening was performed for antibodies with specificities for the antigenic components of the different reference systems for *A. israelii* in concentrated rabbit antibody pools to formalin-treated whole cells of the related bacteria (*A. viscosus*, serotype 1 [ATCC 15987]; *A. viscosus*, serotype 2 [ATCC 19246]; *A. naeslundii* [ATCC 12104]; *A. odontolyticus*, serotype 1 [ATCC 17982]; *A. odontolyticus*, serotype 2 [WVU 482]; *A. propionica*, serotype 1 [ATCC 14157]; *A. propionica*, serotype 2 [WVU 346]; *P. acnes*, serotype 1 [NCTC 737] and serotype 2 [ATCC 11828]; *P. avidum* [ATCC 25577]; *P. granulosum* [ATCC 25564]; and *N. asteroides* [ATCC 19247]). By comparison with the control plates, no reference antigens of *A. israelii* StAgSL were retained by immunoprecipitation in the intermediate gel by these test sera. Antibodies with specificities for components of the standard preparations of the chemical extracts, StAgHCl and StAgurea of *A. israelii*, were detected in antisera raised against *A. naeslundii* by deflections of the reference precipitins coded HCl1 and urea4 compared to the control plates. Antibodies against the antigenic components urea4 of the antigen standard StAgurea were also revealed in sera raised against *P. acnes*, serotypes 1 and 2. No precipitins were detected in these experiments that indicated the presence of antibodies with specificities to other antigenic components of the standard antigens than the reference antigens. No antibodies with specificity for the components of the different standard antigens of *A. israelii* were detected in rabbit antisera to *C. albicans*, *A. fumigatus*, *Thermoactinomyces vulgaris*, and *Micropolyspora faeni*.

The identification of antibodies in the sera of patients to *A. israelii* was conducted by means of CIE with intermediate gel containing these sera by the standard antigen-antibody system for *A. israelii* based on StAgSL and StAbII as reference.

In sera from nine patients with actinomycosis, precipitating antibodies were detected against this reference standard antigen. The precipitin response varied from patient to patient. The number of retained reference antigens in the intermediate gel containing the serum of the patient ranged from one to four at the time of diagnosis (Table 1). All precipitins were identified in terms of the reference system for *A. israelii*. Antibodies specific for one of the cytoplasmic antigen components of *A. israelii* were present in sera from four patients. Two of these patients exhibited thoracic actinomycosis, and two patients exhibited a cervicofacial form of actinomycosis. This precipitin was identified as antigen coded 7 or 10 (17). The sera from two patients with cervicofacial actinomycosis had antibodies to two of the reference antigens, identified as precipitin coded numbers 7 and 10 and numbers 8 and 10, respectively. Antibodies to four reference antigens, identified as precipitins 6, 7, 8, and 10, were detected in sera from one patient with cervicofacial actinomycosis (Fig. 1). No attempts were made to quantitate the immune responses. No immunoprecipitation reactions were obtained that indicated the presence of free antigens of *A. israelii* in these sera.

Follow-up studies of the sera from six patients revealed that the precipitin responses demon-

TABLE 1. *Serological follow-up of patients with proven actinomycosis*

Patient	Precipitins at time of testing									
	Time of diagnosis		2 months		4 months		6 months		1 year	
	No.	Identified as:	No.	Identified as:	No.	Identified as:	No.	Identified as:	No.	Identified as:
GP	1	10[a]	1	10	1	10	0		ND[b]	
GH	2	8, 10	2	8, 10	1	10	1	10	ND	
MF	1	10	1	10	1	10	0		ND	
AÅ	1	7	1	7	ND		0		ND	
SK	2	7, 10	1	10	1	10	1	10	ND	
NZ	4	6, 7, 8, 10	4	6, 7, 8, 10	4	6, 7, 8, 10	4	6, 7, 8, 10	5	6, 7, 8, 9, 10

[a] Precipitin codes, see text.
[b] ND, Not done.

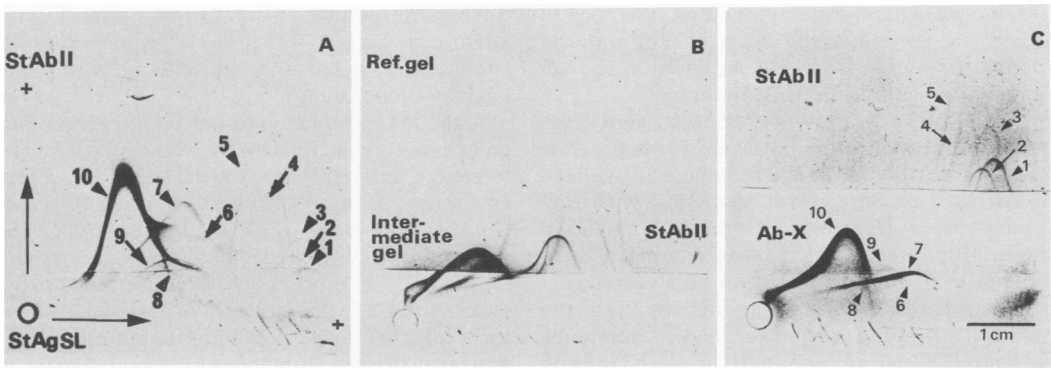

FIG. 1. *CIE with intermediate gel using a cytoplasmic fraction of A. israelii (StAgSL) as antigen and rabbit anti-A. israelii serum (StAbII) as reference. (A) The standard antigen-antibody system for A. israelii by means of CIE; (B) positive control plate with the reference antibody standard in the intermediate gel; (C) serum from a patient with proven actinomycosis. The patient's precipitin response is indicated by the precipitates coded 6, 7, 8, 9, and 10 in the intermediate gel.*

strable at the time of diagnosis were gradually lost in the course of time after substantial clinical improvement following treatment. Six months after establishment of the diagnosis, the sera which initially contained antibodies to one of the reference antigens had lost their precipitating reactivity. The two patients with antibody specificities to two reference antigens had lost antibodies with specificity for antigens coded 7 and 8, respectively. The sera from the remaining patient maintained the initial precipitin pattern. A follow-up study of consecutive sera from this patient during exacerbations, covering a period of 1.5 years, showed an increased number of precipitating antibodies to *A. israelii*. After 1 year, antibodies with specificity for the reference antigen component coded 9 were also demonstrable.

In control experiments, neither the sera from blood donors nor from patients with tuberculosis, nocardiosis, deep candidosis, chronic acnes vulgaris, or chronic periodontal disease precipitated the reference standard antigen.

DISCUSSION

CIE with intermediate gel (1) in conjunction with a reference antigen-antibody system for *A. israelii* (17) appeared to be useful for serological taxonomic studies of *A. israelii* and for the study of circulating antibodies to *A. israelii* in human actinomycosis.

Analysis of the precipitin reactions, obtained by CIE against standard antigens of *A. israelii* with antisera to some related bacteria, supported current classification of a separate serological group of *A. israelii* (5, 16, 22–24). The serological reaction of the components of the cytoplasmic standard antigen of *A. israelii* displayed species specificity. The distinction of a species-specific serogroup of *A. israelii* by precipitation tests seems to be less apparent with other antigenic materials of *A. israelii*. In the present study, this was indicated by the cross-reactivities found between antisera produced against whole cells of type strains of *A. naeslundii* and *P. acnes* and some components of the whole cell-associated antigens of *A. israelii*, obtained by hydrochloric acid, trichloroacetic acid, and urea extractions. The findings of common antigens between *A. israelii* and *A. naeslundii* are in agreement with those found previously in agar gel diffusion studies with supernatant culture fluid as the antigen. Precipitating antigens, derived by acid extraction of cells of *A. israelii*, include species-specific antigens and cross-reacting components. Werner et al. (25) recently found that rabbit antisera against *Propionibacterium* (*Corynebacterium*) *acnes* strains in double immunodiffusion tests formed one or two precipitation lines with *A. israelii* polysaccharide antigens, obtained by formamide extraction, and the clear supernatant of autoclaved whole cell suspensions of *A. israelii*. These findings are in agreement with the cross-reactions found in the present study between anti-*P. acnes* sera and one component of the standard preparation of urea extract, StAgurea.

The precipitating components of the cytoplasmic standard antigen of *A. israelii* appeared specifically diagnostic for *A. israelii*. They also possessed immunochemical properties which made them more suitable for CIE studies (17) than the components of the standards extracted from whole cells of *A. israelii*. The antigen-antibody system for *A. israelii*, based on the cytoplasmic antigen standard and the standard antiserum, raised against crude cell lysates of *A.*

israelii, was therefore employed as reference in assays to estimate the humoral immunoresponse to *A. israelii* in patients with actinomycosis caused by this organism.

Using the technique of CIE with sera from patients in the intermediate gel, it was possible to detect antibodies to the reference antigens of *A. israelii* in sera from patients with actinomycosis at the time of diagnosis. Analysis of the immunoprecipitation patterns obtained indicated that there appeared to be a correlation between the time of active disease and the humoral immune response to the reference antigens. Thus, in one patient with rapidly fulminating thoracic actinomycosis with a known history of disease for about 2 months and a short period of remission after successful treatment, the immune response was surprisingly weak at the time of diagnosis, with antibodies to only one component of the reference antigens of *A. israelii* compared to that evoked in sera from a patient with a persistent active cervicofacial actinomycosis covering a period of 1.5 years, which during that time developed antibodies to six components of the reference system.

Although significant differences in the immunoresponse of individual patients may occur, these observations provide evidence for an increased humoral immunoresponse to the reference antigens of *A. israelii* in chronic actinomycotic infections. The presence of one or two precipitins could indicate any form of actinomycosis. The examination of serial serum specimens provided information about the clinical course of the disease. After successful treatment there was a reduction in the number of precipitins.

No titration of the positive sera with *A. israelii* precipitins was made in the present study. Titration might be useful in following the clinical course of the disease. It would be expected that titration would reveal a lower level of antibody prior to the time of disappearance of the precipitin reaction and, therefore, would provide an earlier guide for response to therapy.

The role of the humoral antibodies in actinomycosis is still unclear. It is assumed that infections with *A. israelii* raise protective humoral antibodies to prevent dissemination of the infection to parietal organs. Mere colonization with *A. israelii*, which is a common commensal of tonsillar crypts in chronic tonsillitis (13) or of periodontal pockets in chronic periodontal disease (K. Holmberg, Arch. Oral Biol., in press), does not seem to evoke detectable humoral responses to the reference antigens of *A. israelii* as indicated by the absence of precipitating antibodies in sera from patients with advanced periodontal lesions from which *A. israelii* had been isolated and in sera from healthy blood donors.

Although extensive studies were carried out, cross-reacting antibodies with specificity for the reference antigens were not detectable in serum specimens from patients with other diseases than actinomycosis. This contrasts with the considerable cross-reactions with *A. israelii* found with heterogeneous sera from other diseases, such as tuberculosis, nocardiosis, streptococcal infections, and some fungal infections, and also in sera from healthy blood donors in previous serological surveys of actinomycosis (11, 15). However, these studies used as antigen chemical fractions of *A. israelii* (15) and crude antigens of *A. israelii* prepared by sonic treatment, formamide extraction of cells or acetone precipitation of supernatant fluids from broth cultures (17) in conventional complement fixation, hemagglutination, and gel diffusion tests.

CIE with intermediate gel using a monospecific standard antigen-antibody system for *A. israelii* as reference permitted the serodiagnosis of actinomycosis. The value of the test rests on the monospecificity of the reference antigens of *A. israelii* and the fact that the primary production of antibodies is directed to these antigens in the course of natural infections. The high sensitivity and the resolving power of CIE permits the determination of antibody specificities in complex sera to the reference antigens of the crude antigen mixtures. The test seems to be a promising method for routine laboratory practice, with a major advantage being that antigen production is easy and purification of the reference antigens is unnecessary. The test fulfills the need for efficiency of a laboratory test with regard to sensitivity and specificity. The possibility for standardization would be introduced by the test. A standardized test used in prospective clinical trials would yield comparable information from different laboratories and, in turn, clarify the problem concerning the effectiveness of the test in diagnostic *A. israelii* serology.

LITERATURE CITED

1. Axelsen, N. H. 1973. Intermediate gel in crossed and in fused rocket immunoelectrophoresis. Scand. J. Immunol. **2**:71–77.
2. Axelsen, N. H., M. Harboe, O. Closs, and T. Godal. 1974. BCG antibody profiles in tuberculoid and lepromatous leprosy. Infect. Immun. **9**:952–958.
3. Axelsen, N. H., C. H. Kirkpatrick, and R. H. Buckley. 1974. Precipitins to *Candida albicans* in chronic mucocutaneous candidiasis studied by crossed immunoelectrophoresis with intermediate gel. Clin. Exp. Immunol. **17**:385–394.
4. Bowden, G. H., and J. M. Hardie. 1970. Commensal and

pathogenic *Actinomyces* species in man, p. 277-299. *In* G. Sykes and F. A. Skinner (ed.), Actinomycetales, characteristics and practical implications. Academic Press Inc., New York.
5. Brock, D. W., and L. K. Georg. 1969. Determination and analysis of *Actinomyces israelii* serotypes by fluorescent-antibody procedures. J. Bacteriol. **97**:581-586.
6. Brock, D. W., and L. K. Georg. 1969. Characterization of *Actinomyces israelii*, serotypes 1 and 2. J. Bacteriol. **97**:587-589.
7. Brock, D. W., L. K. Georg, J. M. Brown, and M. D. Hicklin. 1974. Actinomycosis caused by Arachnia propionica. Am. J. Clin. Pathol. **59**:66-77.
8. Cummins, C. S. 1970. *Actinomyces israelii*, type 2, p. 29-34. *In* H. Prauser (ed.), The Actinomycetales. VEB Gustav Fischer Verlag, Jena.
9. Georg, L. K. 1970. Diagnostic procedures for the isolation and identification of the etiologic agents of actinomycosis, p. 71-80. *In* Proceedings Int. Symp. Mycoses. World Health Organization, Washington, D.C.
10. Georg, L. K., and C. M. Coleman. 1970. Comparative pathogenicity of various *Actinomyces* species, p. 35-45. *In* H. Prauser (ed.), The Actinomycetales. VEB Gustav Fischer Verlag, Jena.
11. Georg, L. K., R. M. Coleman, and J. M. Brown. 1968. Evaluation of an agar gel precipitin test for the sero-diagnosis of actinomycosis. J. Immunol. **100**:1288-1292.
12. Georg, L. K., G. W. Robertstad, and S. A. Brinkman. 1964. Identification of species of *Actinomyces*. J. Bacteriol. **88**:477-490.
13. Gruner, O. P. N. 1969. Actinomyces in tonsillar tissue. Acta Pathol. Microbiol. Scand. **76**:239-244.
14. Holm, P. 1950. Studies on the aetiology of human actinomycosis. Acta Pathol. Microbiol. Scand. **27**:736-751.
15. Holm, P., and J. B. Kwapinski. 1959. Studies on the detection of *Actinomyces* antibodies in human sera by using pure antigenic fractions of *Actinomyces israelii*. Acta Pathol. Microbiol. Scand. **45**:107-112.
16. Holmberg, K., and U. Forsum. 1973. Identification of *Actinomyces*, *Arachnia*, *Bacterionema*, *Rothia* and *Propionibacterium* species by defined immunofluorescence. J. Bacteriol. **25**:834-843.
17. Holmberg, K., C.-E. Nord, and T. Wadström. Serological studies of *Actinomyces israelii* by crossed immunoelectrophoresis: standard antigen-antibody system for *A. israelii*. Infect. Immun. **12**:387-397.
18. Højby, N., and N. H. Axelsen. 1973. Identification and quantitation of precipitins against *Pseudomonas aeruginosa* in patients with cystic fibrosis by means of crossed immunoelectrophoresis with intermediate gel. Acta Pathol. Microbiol. Scand. Sect. B **81**:298-308.
19. King, S., and E. Meyer. 1963. Gel diffusion technique in antigen-antibody reactions of *Actinomyces* species and "anaerobic diphtheroides." J. Bacteriol. **85**:186-190.
20. Lambert, F. W., J. M. Brown, and L. K. Georg. 1967. Identification of *Actinomyces israelii* and *Actinomyces naeslundii* by fluorescent-antibody and agar gel diffusion techniques. J. Bacteriol. **94**:1287-1295.
21. Slack, J. 1942. The source of infection of actinomycosis. J. Bacteriol. **43**:193-209.
22. Slack, J. M., and M. A. Gerencser. 1966. Revision of serological grouping of *Actinomyces*. J. Bacteriol. **91**:2107.
23. Slack, J. M., and M. A. Gerencser. 1970. Two new serological groups of *Actinomyces*. J. Bacteriol. **103**:265-266.
24. Slack, J. M., S. Landfried, and M. A. Gerencser. 1969. Morphological, biochemical, and serological studies on 64 strains of *Actinomyces israelii*. J. Bacteriol. **97**:873-884.
25. Werner, H., G. Rintelen, and G. Böhm. 1974. Untersuchungen zur Frage der Antigen-gemeinschaft zwischen *Actinomyces israelii* and *Corynebacterium acnes*. Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **226**:264-271.