THE COMPARISON OF FOUR STRAINS OF COCCIDIOIDES IMMITIS WITH DIVERSE HISTORIES[1])

by

LORRAINE FRIEDMAN [2]) and C. E. SMITH

(Naval Biological Laboratory, Department of Bacteriology and School of Public Health, University of California, Berkeley, California, U.S.A.)

(1.9.1955)

Only 40 per cent of human beings infected by the fungus *Coccidioides immitis* develop clinical symptoms. About one per cent of adult white males and ten per cent of negro males infected with manifest symptoms progress to the disseminated, progressive extrapulmonary coccidioidal granuloma which is often fatal (1). The cause of dissemination is unknown, but among the theories considered is variation in the pathogenicity of the strains. Recently a strain was submitted to this laboratory which was isolated from a rapidly disseminating and fatal case of coccidioidomycosis. The morphology in culture and the virulence of this strain in laboratory hosts has been compared with other strains of widely differing histories.

Methods

Source of strains — Strain Soil 3a was cultured fifteen years ago directly from the earth where a group of University biology students were infected while digging a rattlesnake out of a ground squirrel hole (2). None of their infections disseminated. While the identity of the strain was established by animal inoculation of parallel cultures, the lineal line of the experimental culture had never been

[1]) This work was supported in part by a contract between the University of Calif., Department of Bacteriology and the Office of Naval Research and part under the sponsorship of the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board and was supported by the Office of the Surgeon General, Department of the Army.

The opinions contained in this report are not to be construed as reflecting the views of the Navy Department or the Naval Service at large (Article 1252, U.S. Navy Regulations, 1948).

[2]) Present address: Department of Microbiology, Tulane University, School of Medicine, New Orleans, Louisiana.

The authors gratefully acknowledge the photographic service of Mr. WILLIAM F. HETRICK and the technical services of Mr. ROBERT J. BERMAN.

passed through laboratory animals. There had been a minimum of thirty transfers on media during this time.

Strain Euphrat was recovered three years ago from a localized cutaneous lesion of a patient of Dr. EUGENE FARBER. The patient's serum fixed complement only in a 1 : 2 dilution, and with this favorable prognostic evidence (3) the lesion was excised. There has been no recurrence.

Strain 46 was isolated twenty-one years ago from a patient who died of a disseminated coccidioidal infection.

The Johnson strain was supplied us in 1954 by Dr. MARSHALL FIESE from a patient of the Fresno Veterans' Administration Hospital with a fulminating coccidioidal infection. His serum fixed complement in a titer of 1 : 256 and he died in two months. There had been only two transfers on media since isolation from the patient.

Preparation of inocula — Material was harvested from petri dish cultures (1 per cent glucose, 0.5 per cent yeast extract and 2 per cent agar) which had been incubated at 35° C until dry.

Assay of virulence — Twenty-five white male mice, ten weeks old, were inoculated intraperitoneally with one milliliter of a suspension containing 100 viable particles as determined by triplicate pour plates at the time of inoculation. The relative virulence of the strains was compared by the percentage of animals dead thirty days after inoculation. The relation of time to mortality as an expression of virulence has been described by FRIEDMAN, SMITH & GORDON (4).

In the case of strain 46, higher dosages, up to one million particles, were also given. Strain 46 was assayed also following an attempt to increase the virulence by twelve intraperitoneal passages through mice. Passage was from mouse to mouse without intervening culture on artificial media and extended over a period of six months.

Results

At the 100 particle dose level, strains Soil 3a, Johnson, and Euphrat were equally lethal for mice (76, 64 and 72 per cent dead, respectively, thirty days after inoculation whereas a strain 46 appeared avirulent (table 1). However, at autopsy ninety days after inoculation, 68 per cent of the animals which received strain 46 showed gross lesions (typified by figure 1). All of the mice which were inoculated with the other three strains also were infected.

Not until the dosage of strain 46 was increased to a million particles did the rate of dying approximate that of the animals inoculated with 100 particles of the other strains. Lesions in the animals which died following the high dose of strain 46 were similar to those seen in animals with the lower dosages of the other three strains (figures 2 and 3).

Passage of strain 46 through mice did not alter the virulence of that strain for mice.

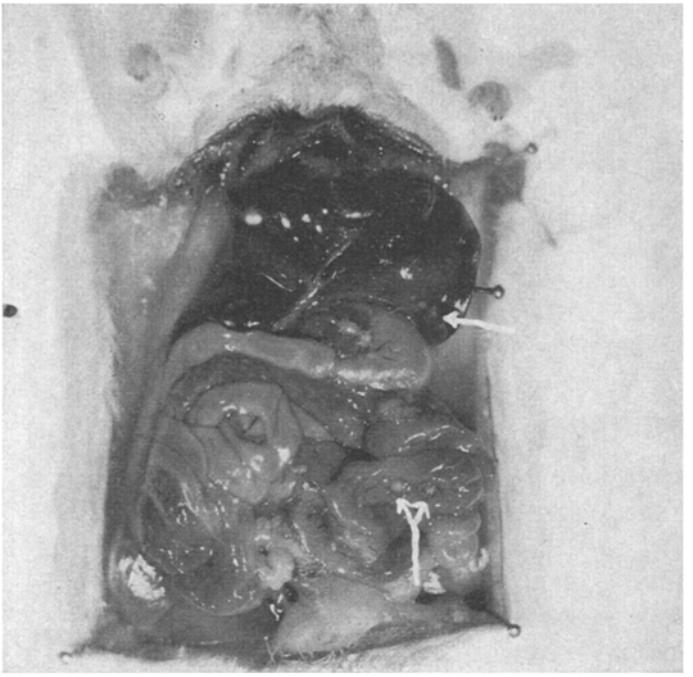

Figure 1. A mouse which was inoculated with 100,000 particles of strain 46 and sacrificed after 90 days. The liver is pinned back to expose the characteristic fibrotic lesions.

Figure 2. The lungs of a mouse which died 20 days after inoculation with one million particles of strain 46.

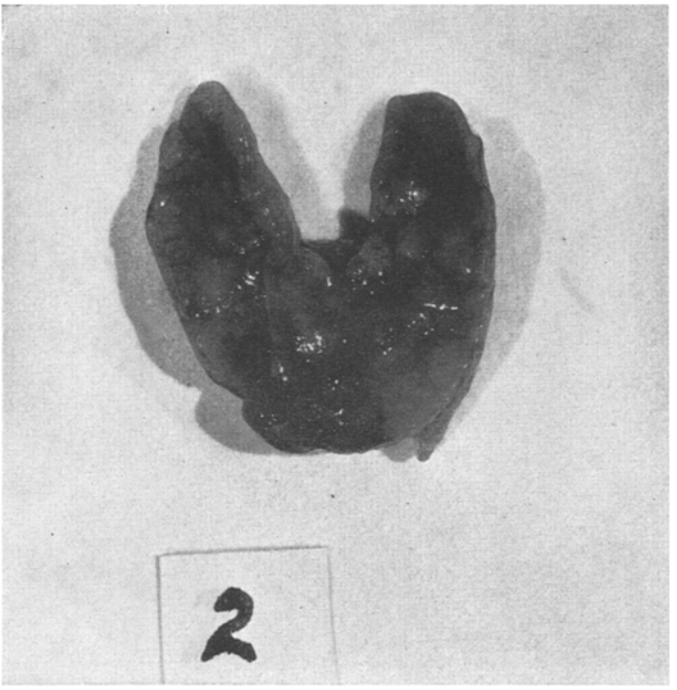

Figure 3. The lungs of a mouse which died 33 days after inoculation with 10 particles of strain Soil 3a.

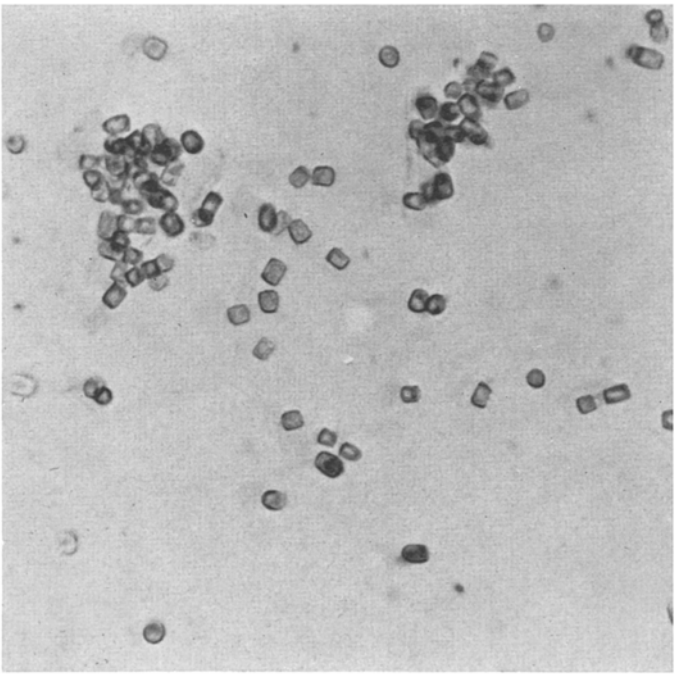

Figure 4. Microscopic preparation of a culture of strain Euphrat. Hyphae have converted entirely to classical arthrospores. (× 440).

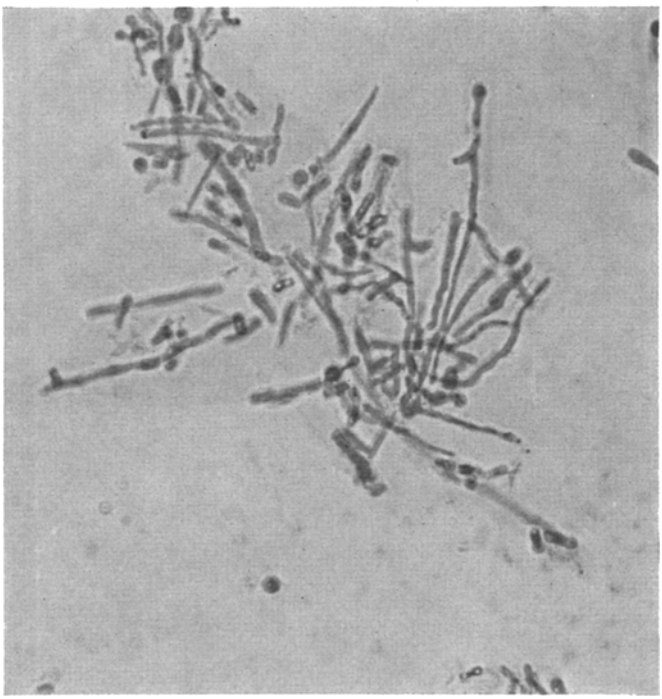

Figure 5. Microscopic preparation of a culture of strain 46, the same age as the cells of figure four. Hyphae did not convert to classical arthrospores even after further incubation. (× 440)

TABLE I History and virulence of cultures

Strain	Date of isolation	Clinical history	Virulence for mice, percentage of animals dead 30 days after inoculation with 100 viable particles
Johnson	Jan '54	Coccidioidal granuloma, fatal within 6 months	64
46	1933	Coccidioidal granuloma, fatal	0
Soil 3a	1939	None, isolated from soil	72
Euphrat	1951	Coccidioidal granuloma, dissemination limited to cutaneous area, patient recovered	76

The sporulating capacities in culture differed markedly. Soil 3a, Euphrat (figure 4) and Johnson produced spores of classical morphology, although the quantity produced by the Johnson strain was sparse. Strain 46 spores were not only low in quantity but aberrant in morphology (figure 5).

Summary

By the criterion of lethality in mice, a strain of *Coccidioides immitis* recently isolated from a most devastating form of human coccidioidomycosis was of no greater virulence than another isolated from a much less severe case or even one cultured from soil fifteen years ago and which presumably had caused seven self-limited, benign cases of primary coccidioidomycosis. There was no enhancement of virulence to mice by twelve passages of a relatively avirulent strain which had been recovered twenty-one years ago from a fatal human case.

Conclusions

There appears to be no correlation between severity of human coccidioidomycosis and virulence of the etiologic strain for mice. Possibly dosage of inoculum and certainly host factors play determining roles in the human infection spectrum.

Literature

SMITH, C. E., BEARD, R. R., WHITING, E. G., & ROSENBERGER, H. G. (1946) Varieties of coccidioidal infection in relation to the epidemiology and control of the diseases. A. J. P. H. **36**, 1394—1402.

DAVIS, B. L., Jr., SMITH, R. T., & SMITH, C. E. (1942) An epidemic of coccidiodal infection (coccidioidomycosis). J. A. M. A. **118**, 1180—1186.

SMITH, C. E., SAITO, M. T., BEARD, R. R., KEPP, R. M., CLARK, R. W., & EDDIE, B. V. (1950) Serological tests in the diagnosis and prognosis of coccidioidomycosis. Am. J. Hyg. **52**, 11—21.

FRIEDMAN, L., SMITH, C. E., & GORDON, L. E. (1955) The assay of virulence of Coccidioides in white mice. J. Inf. Dis. *To be published.*