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Isolation of *Coccidioides Immitis* (Stiles) from the Soil.

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The epidemiological studies on coccidioidal granuloma, recently summarized by Beck,¹ suggest the soil and the vegetation as the probable source of the fungus infection. The peculiar geographic distribution of the disease both in man and animals to the central and southern sections of the State of California with a predominance of the cases in Kern and Los Angeles Counties supports this contention. Although repeated attempts have been made to find the organism in nature the examinations, made with the aid of the customary bacteriologic technique, have thus far yielded negative results. The relatively slow growing atypical fungi are, as a rule, rapidly overgrown by the concomitant bacteria present in the soil and vegetations. Little progress could be made until a procedure had been developed which would concentrate, possibly enrich the stage of the mold (chlamydo-spore) supposed to exist in nature. In the course of detailed metabolism studies it was found that *Coccidioides immitis* satisfies its nitrogen and carbon requirements from relatively simple compounds. The medium, which gives selective cultures, has the following composition: Ammonium chloride 1 part, sodium acetate 1 part, equal mixture of KH_2PO_4 and K_2HPO_4 0.4 part and magnesium sulfate 0.01 part; distilled water 100 parts. While the majority of bacteria grow slowly or very poorly, *Coccidioides*, certain *Blastomyces* and *Aspergillus* varieties develop exceedingly well in the nutrient solution. Bacterial multiplication may be entirely suppressed and the purification of the fungus greatly facilitated by the addition of acriflavine in a concentration of 1:25,000. For several years the medium has been employed in the examination of clinical specimens (sputum, pus, blood, autopsy specimens, etc.) suspected to contain the mold. Samples of sputum, which contained very few typical double contoured fungoid cells yet teeming with the usual respiratory and buccal flora, gave pure cultures of *Coccidioides immitis* in the dye culture medium.

Since *Coccidioides* survives the exposure to a 30-35% NaCl solution for upwards to 3 to 4 hours, a method has been developed which permits the concentration of the fungus spores from large samples of soil or vegetation. The procedure, which has repeatedly

¹ State of California Dept. of Public Health Special Bull., 1931, 57, 19.

yielded the pathogenic fungus from specimens of earth collected around the sleeping quarters at a ranch near Delano, Kern County, is as follows: The sample of dirt is mixed with a small amount of brine and triturated into a thick paste which in turn is permitted to percolate into a tall, narrow cylinder containing 30% salt solution. As the particulate matter passes through the long column of brine the bacterial and fungus spores are set free, and rise slowly to the surface of the liquid. After an exposure of the soil to the action of the brine for 3 hours the supernatant fluid is decanted and diluted one-half with sterile water and centrifuged. The sediment is either cultured in the selective medium or inoculated subcutaneously into guinea pigs.

Positive findings on the soil of the Delano ranch had been anticipated on account of the occurrence of 4 cases of Coccidioidal granuloma among the Filipino working crew (1, p. 25). The guinea pigs, injected subcutaneously with the soil sediment, developed either a generalized infection with miliary localization in the lungs, purulent epididymitis and lymphadenitis or merely temporary small abscesses at the site of inoculation. The pus contained the characteristic double contoured capsules with the endospores. Obviously, the mold, isolated from the soil, is highly virulent, and infects on primary inoculation. However, it is not unlikely that the variable susceptibility of the guinea pigs may, in part, have been responsible for the failure to detect the fungus in a few other samples. Experiments are now in progress to improve the pathogenicity tests and to demonstrate directly by special cultures or other methods, the resting stage, which permits the mold to survive for months in a desiccated soil specimen.