SERUM GLYCOPROTEINS IN RATS WITH EXPERIMENTAL COCCIDIOIDOMYCOSIS 1)

by

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INTRODUCTION

Significant increases in the levels of glycoproteins in the serum have been reported in a variety of infectious and neoplastic diseases in man as well as in several experimentally-induced pathological states in laboratory animals (1). The effect of mycotic infections on serum glycoproteins has not been studied in detail. The purpose of this investigation was to evaluate total glycoproteins, seromucoids, haptoglobins and specific antibodies during the course of experimental coccidioidomycosis in rats.

MATERIALS AND METHODS

Fungus: Coccidioides immitis, Silveira strain, in a logarithmic growth phase was cultured in Sabouraud dextrose broth at 37°C on a rotary shaker for seven days. The fungus was concentrated by centrifugation, washed three times with sterile .85% saline, and ground thoroughly in a Ten Broek tissue grinder to obtain a uniform suspension. The final suspension in saline was adjusted to 50% by volume.

Animals: Rats (Wistar strain), bred in our laboratory, were three to five months old at the start of the experiment. The weight of the males varied from 150 to 400 g and that of the females from 150 to 280 g. They were allowed laboratory chow and water ad libidum.

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Seventy rats of both sexes were distributed into seven groups. Two groups were retained as controls and five were inoculated with 0.5 ml of the suspension of *C. immitis*. Three days later the animals in one of the control groups and in one of the inoculated groups were exsanguinated under ether anesthesia. The other infected groups were similarly exsanguinated at one week, two weeks, four weeks, and 16 weeks after inoculation. The remaining group of control rats was exsanguinated at the same time as the 16 week group. All the sera were separated within a few hours after bleeding and stored at $-20^\circ$ C until used for the analyses. Blood was not obtained from six rats, which died prior to their sacrifice date.

All the animals, including the controls, were autopsied and examined for evidence of infection. Portions of the spleen, liver, lungs, and kidneys from each rat were cultured on Sabouraud dextrose agar.

Chemical analysis: Standard procedures for determining total protein (2), total glycoprotein (as hexose) (3), seromucoid protein (4), seromucoid hexose (3), and haptoglobin (5) were followed, but the amounts of materials were altered in some studies to conserve serum. Each determination was performed in duplicate.

The mean, standard error of the mean, t, and probability value were determined (6). The standard error of the mean for each figure is included in the tables.

Serological tests: The antigen used in the tube precipitin test (7) for *C. immitis* was obtained from Microbiological Consulting Service, Van Nuys, California and the one used in the complement fixation test (7) was prepared in the laboratory from a four-fold dilution of culture filtrate of *C. immitis*, Silveira strain, cultured for 30 days at $37^\circ$ C in Sabouraud dextrose broth. Sera were diluted one to four prior to testing for complement fixing antibodies.

Results

Table I presents the initial and final weights and the results of the complement fixation test, precipitin test and cultures.

No trend in weight deviations was apparent in the average weights of rats in the infected groups when compared with the expected average of rats in the control groups. Complement fixing antibody could be detected as early as three days after infection and was present in the sera of all animals after one week and in most after two weeks. By the fourth week, however, this antibody could only be detected in one of eight animals and in none of the animals surviving to sixteen weeks. Titers ranged from 8 to 410 with the highest occurring one week after infection. The precipitins, essentially, paralleled the complement fixing antibodies. The highest titer found was 8. Neither antibody test was positive with sera from any control animal.

Random sampling of the various organs obtained at autopsy