

Serum Protein Patterns in Experimental Coccidioidomycosis.* (27836)

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Electrophoretic separation of serum proteins has become an important means of demonstrating the presence and progress of several disease states. A survey of changing levels of serum protein fractions provides a suitable beginning for studies of a host's metabolic response to a disease. The following experiment was undertaken to determine if significant changes occur in serum protein fractions of mice infected with *Coccidioides immitis*.

Methods. Arthrospore and hyphal fragments of *Coccidioides* were washed with isotonic saline from Sabouraud's dextrose agar after 6-weeks growth at 37°C. The washings were plated and the colonies counted for viability estimation. The particle suspensions were then adjusted to a concentration of 200 viable particles in a volume of 50 μ l and used for inoculum.

Fifty male and 50 female Namru mice of uniform age and weight were anesthetized with ether and inoculated intranasally with a total of 400 viable particles in two successive inoculations of 200 particles each. Inoculated mice were caged in groups of 10, and each cage was paired with a cage of 4 uninoculated mice of the same sex.

Sampling was started the second day after inoculation. All mice from one cage of inoculated males and those from the corresponding control cage were anesthetized by intraperitoneal injections of thiamylal sodium, after which blood was drawn from their hearts. The process was continued with successive cages every second day thereafter, the sex of the sampled mice being alternated. The number of analyses varied from one sampling to the next because hemolyzed samples were discarded and because some mice developed fatal coccidioidal infection before arriving at their turn to be sampled.

Ten microliters of clear serum were sepa-

rated electrophoretically on paper strips in Durrum type cell with sodium Veronal buffer (pH = 8.6; μ = .075) for 17 to 18 hours. Total serum protein was estimated refractometrically(1).

The amount in grams of each serum protein fraction in 100 ml of serum was determined by multiplying the percentage of each fraction with the grams percent of total protein. The resulting data from each fraction and from total protein were subjected to regression analysis against time, then summarized by a variance analysis of each regression(2).

Results. The serum protein patterns contained 4 distinct fractions—albumin, alpha globulin, beta globulin, and gamma globulin (Fig. 1). The alpha globulin separated was alpha-2. The alpha-1 fraction was too closely associated with albumin to be estimated quantitatively, as shown by the last 2 patterns in Fig. 1.

The successive serum protein patterns illustrated in Fig. 1 show an obvious increase in alpha and beta globulin and a decrease in albumin with time. These changes were consistent from mouse to mouse, whereas the slight change in gamma globulin was not. The variation in the latter fraction may be considered due to experimental error.

The rate at which total protein and each protein fraction changed with time is shown by Fig. 2. This figure also indicates that male mice, which were sampled on the 2nd, 6th, 10th, and 14th days after inoculation, had higher levels of total protein, albumin and alpha globulin than female mice sampled on the remaining days. Beta globulin was only slightly higher in male mice, while gamma globulin did not vary with sex. The sex variation in mouse serum proteins, which became obvious after a large amount of data was accumulated, made it necessary to adjust the observations to a common mean of each dependent variable.

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Once the data were adjusted for sex variation, the time of first change in any fraction,

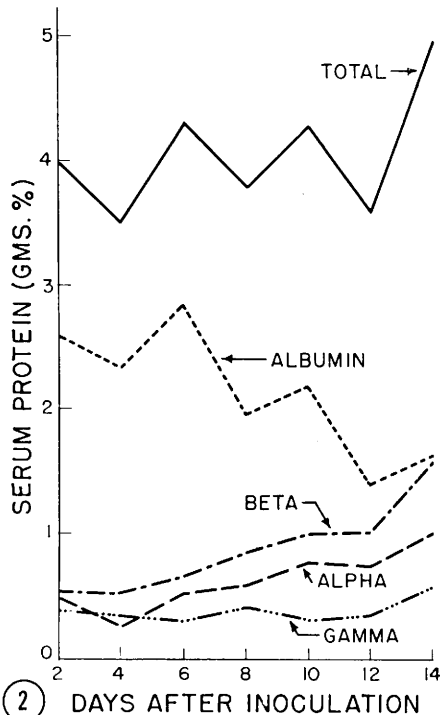
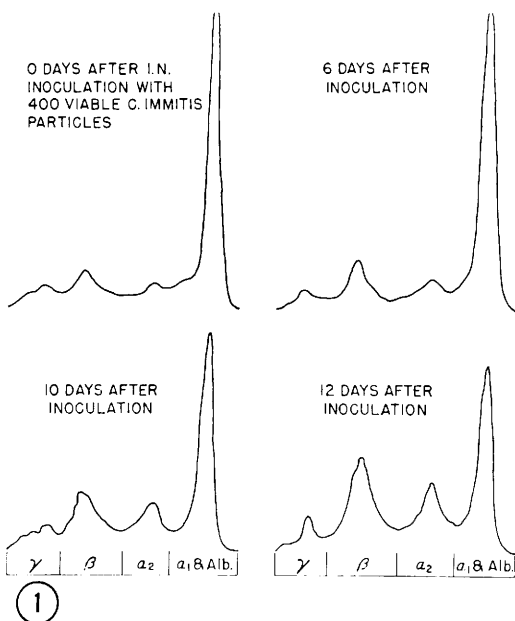


FIG. 1. Sequential serum protein patterns from mice inoculated with *Coccidioides*.

FIG. 2. Changes in mean total protein and serum protein fractions from mice inoculated with *Coccidioides*.

TABLE I. Regression and Correlation Coefficients of Total and Serum Protein Fractions against Time and Variance Analyses of the Regressions.

Protein fraction	Degrees of freedom	Mean square	Coefficient	
			Regression	Correlation
Total protein	1	1.65*	.046	.361
Error	55	.20		
Albumin	1	6.04*	-.088	.726
Error	55	.10		
α -globulin	1	1.80*	.052	.726
Error	55	.03		
β -globulin	1	4.42*	.075	.842
Error	55	.03		
γ -globulin	1	.02	.005	.140
Error	55	.02		

* F values were significant at $P < .01$.

or in total protein, became more obvious than is apparent in Fig. 2. Alpha and beta globulin began to increase between the 4th and 6th day after inoculation. Albumin started its decline between the 6th and 8th days. Total protein appeared to climb steadily throughout the experimental period. Coincident with these measurements was the appearance of gross lesions in the lungs on day 4 and similar lesions in the liver on day 8 in most of the mice observed.

The data summarized in Table I further support the conclusions that may be drawn from Fig. 1 and 2. A highly significant ($P < .01$) amount of the variation in levels of total protein and all fractions except gamma globulin can be explained by the progression of the infection with time. The rate of change was greatest in albumin, but degree of linear association with time was greatest in beta globulin.

Discussion. In the acute stage of tuberculosis in experimentally infected guinea pigs serum albumin was found to decrease and the alpha-1 and gamma globulins increased while total protein and beta globulin did not change(3). By comparison, the serum from *Coccidioides* infected mice also showed a decrease in albumin but an increase in total protein, beta globulin and alpha-2 globulin. Gamma globulin remained unchanged.

A decrease in serum albumin has been associated with hepatic disease in man(4). The appearance of coccidioidal liver lesions at the

time mouse serum albumin levels dropped reinforces such observations and further suggests that a drop in albumin may herald spread of the disease beyond thoracic viscera.

It has been suggested that the change in serum protein levels of tubercular humans is the product of inflammation(5). The observed increases in the alpha-2 and beta globulins of mice may reflect degrees of tissue destruction or developing hypersensitivity. Since the levels of the 2 fractions rose steadily as the disease progressed and through the period when mice began to die, the tissue destruction thesis seems acceptable.

The lack of increase in gamma globulin may reflect the acute morbidity or a failure of immune response, leading to high death rate.

Since the data are incomplete with respect to measurements of serum volume, no explanation can be offered for the steady climb in total protein.

The consistency of response suggests that change in serum protein levels will serve as a

good measure of disease progress in *Coccidioides* infected mice.

Summary. Serums from mice inoculated intranasally with *Coccidioides* were separated electrophoretically. Total protein and the alpha-2 and beta globulin fractions increased significantly with development of disease, while albumin decreased. Gamma globulin remained constant. Increases in alpha-2 and beta globulins began about the 5th day. Albumin began to decline about the 7th day after inoculation.

1. Sunderman, F. W., *J. Biol. Chem.*, 1944, v153, 139.

2. Ostle, B., *Statistics in Research*, Iowa State Coll. Press, Ames, 1954.

3. Weimer, H. E., Bell, R. T., Froman, S., Nishihara, H., Rice, E. H., *Proc. Soc. Exp. Biol. and Med.*, 1960, v105, 300.

4. Dunn, W. L., Pearce, R. H., *Can. Med. Assn. J.*, 1961, v84, 272.

5. Bovornkitti, S., *Am. Rev. Resp. Dis.*, 1962, v85, 58.

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Lack of Staining of Testicular Tumors by Anti-Sperm and Anti-Testis Antibodies.* (27837)

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Previously we have shown that antibody prepared against a normal cell constituent, myosin, which is unique for muscle cells, reacts only with certain but not all tumors derived from muscle. It reacted with several embryonal rhabdomyosarcomas and not with the so-called adult rhabdomyosarcomas(1). We have now studied the reaction of antisera prepared against testis and spermatozoa to see if there are antigenic components unique to spermatogenic or testicular tissue which are also present in testicular tumors.

That antigenic substances are present in sperm and testicular tissue which are differ-

ent from substances present in other tissues such as kidney has been demonstrated by Freund *et al.*(2). They were able to produce aspermatogenesis and testicular lesions by injecting guinea pigs with sperm or testis tissue in Freund's adjuvants. Injection of other tissues did not produce the lesions.

Materials and methods. Human tissues were obtained at surgery or at necropsy. Diagnoses were made by the Department of Pathology at this Institute (courtesy of Dr. John Pickren).

Normal human semen was obtained from the sperm viability clinic. These samples were treated according to the procedures described by Weil(3), the sperm cells and seminal fluid were pooled separately and frozen.

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