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.

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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 spermatic or testicular tissue which are also present in testicular tumors.

That antigenic substances are present in sperm and testicular tissue which are different 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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TABLE I. Staining Reactions of Various Human Tissues with Anti-Sperm, Anti-Testis and Anti-Seminal Fluid Sera.

	Sperm cells (smear or in testis)	Germinal epithelium in normal testis	Normal tissues: Liver, kidney, adrenal, spleen	Testicular tumors: 4 seminomas, 3 embryonal carcinomas, 2 chorioepitheliomas, mixed tumor
Anti-sperm serum				
Absorbed with rat liver	+	—	Connec. tis.* only	Connec. tis.* only
" " human liver	+	—	—	—
Anti-testis serum				
Absorbed with rat liver	+	+	Connec. tis.* only	Connec. tis.* only
" " human liver	+	+	—	—
Anti-seminal fluid				
Absorbed with rat liver	+	—	—	—
" " human liver	+	—	—	—

* Connective tissue.

Anti-sperm serum was prepared in rabbits by injecting sperm cells in Freund adjuvants. The adjuvant was made by mixing a suspension of 100 mg sperm cells (wet) in 5 ml saline with an equal volume of complete adjuvant. Three rabbits were used. Each received 1 ml of the mixture subcutaneously into each of 4 sites. Injection was repeated weekly for 4 weeks. Starting from the second week 0.5 to 1.0 ml of the sperm suspension (20 mg/ml) without adjuvant was also given intraperitoneally at weekly intervals for 5 weeks. The animals were bled out 1 week after the last injection and the sera pooled.

Anti-seminal fluid serum was prepared by mixing an equal volume of the seminal fluid with complete adjuvants and injecting into 3 rabbits. A total of 1 ml was injected subcutaneously into 4 sites at weekly intervals for 5 weeks. Starting from the 2nd week seminal fluid alone (1.0 ml) was also given intraperitoneally at weekly intervals. Animals were bled 1 week after the last injection and the sera pooled.

Anti-human adult testis serum was prepared by mixing equal portions of a suspension of adult normal testis homogenate (200 mg tissue/ml) with adjuvant and injecting this into 3 rabbits. 0.1 ml was injected into each of 5 sites, 2 intramuscular, 3 subcutaneous, 3 times at monthly intervals. Animals were bled after 5 weeks and then at monthly intervals and the sera pooled. All 3 antisera were capable of immobilizing viable sperm cells and causing agglutination on slide preparations whereas normal rabbit serum was not.

Fluorescent staining procedure. Frozen tissue sections were fixed in acetone in the cold for 9 minutes and washed in saline before treatment with the appropriate antiserum. Sperm cells from a pool kept at $-20°C$ were washed repeatedly (6 times) in an angle head centrifuge ($12{,}800 \times g$). A 2% suspension was prepared and smears made on glass slides. The smears were dried in air fixed in acetone and treated with the appropriate antiserum.

The antisera to be studied were absorbed either with rat liver sediments or human liver sediments according to the procedure previously described(4). Subsequent staining with fluorescein-labeled horse anti-rabbit γ-globulin serum was carried out as described previously(4).

Results and discussion. All antisera against human tissues, *i.e.*, anti-sperm, anti-testis serum, and anti-seminal fluid serum, were absorbed with human liver sediment so that all staining for normal human liver, kidney, adrenal, and spleen was removed. These sera still stained human sperm (smears) (Table I). They also stained the spermatocytes in the central areas of the tubules of all of the several specimens of normal human testes tested. However, only the anti-testis serum was capable of staining the germinal cells which it did weakly. Neither the anti-sperm nor the anti-seminal fluid sera stained the germinal epithelium. Sections of the atrophic testis (showing no spermatogenesis) treated with the above antisera showed no staining. These

results indicate that the anti-testis serum contains antibodies which can stain germinal cells and other components of the testis as well as sperm (Table I). The components are not common to other human tissues such as liver, kidney, adrenal, and spleen since antibodies capable of staining these tissues were removed by absorption with human liver. The anti-sperm and anti-seminal fluid could only stain sperm and spermatocytes.

Actually the anti-seminal fluid serum did not appear to contain antibodies directed against human connective tissues even before absorption with human liver. On the other hand, both anti-sperm and anti-testis antisera did contain such antibodies since they stained liver sinusoids and basement membrane elements of the kidney of the normal human tissues. In these latter studies absorption was performed with rat liver to remove non-specific staining components. Weil *et al.*(3,5) have studied rabbit anti-human seminal fluid and anti-human spermatozoa sera. They found that both antisera appear to react identically with seminal fluid and with sperm by complement fixation, sperm cell agglutination, precipitation tanned cells agglutination and agar diffusion methods.

Lack of staining of tumor tissues. Although the anti-testis sera appeared to show some affinity for adult testicular epithelium, staining of testicular tumors was negative. Studies on 4 seminomas, 3 embryonal carcinomas, 2 chorioepitheliomas and one mixed tumor (a malignant teratoma with embryonal carcinoma and seminoma) were made. None of the tissues were stained by anti-testis antiserum nor with the anti-sperm or anti-seminal fluid sera.

These results show that there are components unique in testis which can be stained with antibody against testis, seminal fluid and sperm but these components were not present at a concentration high enough to be observed in the several testicular tumors tested (Table I).

Summary. The fluorescent antibody technic was employed to determine the presence of normal antigens of sperm, seminal fluid, and testis in various testicular tumors. Ten testicular tumors showed no staining with any of the antisera made to the above normal antigens. These results show that there are components unique in testis which can be stained with antibody against testis, seminal fluid and sperm but these components were not present in the several testicular tumors tested at a concentration high enough to be observed. Some differences were noted between the anti-seminal fluid, anti-sperm and anti-testis sera. The 3 sera reacted with sperm cells. Anti-sperm and anti-testis in addition to staining sperm cells reacted with stromal elements in testis. The latter serum also contained antibodies to germinal epithelium.

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Effect of Partial Hepatectomy on Autologous Tail Tendon Implanted in the Rat Liver.* (27838)

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The ability of the liver to absorb collagen has been demonstrated in two experimental situations(1): first, in cirrhosis of the liver induced in the rat by CCl_4(2,3), "butter yellow"(4), and various diets(5), and second, by implantation of catgut and homologous

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