for the deficient and supplemented group. 2. Other studies indicated that the rates of incorporation of N^{15} glycine into the purines of RNA were lower for the deficient animals. 3. These studies suggest that in a vit. B_{12} deficiency the reduced rate of nucleic acid synthesis in the liver reduced the rate of cell divisions.

1. Kitay, E., McNutt, W. S., and Snell, E. E., *J. Biol. Chem.*, 1949, v177, 993.

2. Shive, W., Ravel, J. M., and Harding, W. M., *J. Biol. Chem.*, 1948, v176, 991.

3. Kitay, E., McNutt, W. S., and Snell, E. E., *J. Bact.*, 1950, v59, 727.

4. Skeggs, H. R., Nepple, H. M., Valentik, K. A., Huff, J. W., and Wright, L. D., *J. Biol. Chem.*, 1950, v184, 211.

5. Schweigert, B. S., Rose, I. A., and Friedkin, M., unpublished data, 1950.

6. Stern, J. R., Taylor, M. W., and Russell, W. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 551.

7. Rose, I. A., and Schweigert, B. S., *Abstracts, Am. Chem. Soc.*, Sept., 1950, p55c.

8. Scheid, H. E., McBride, B. H., and Schweigert, B. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 236.

9. Scheid, H. E., Andrews, M. M., and Schweigert, B. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v78, 558.

10. Schneider, W. C., *J. Biol. Chem.*, 1945, v161, 293.

11. Ogur, M., and Rosen, G., *Arch. Biochem.*, 1950, v25, 262.

12. Davidson, J. N., *Biochem. J.*, 1945, v39, 1ix.

13. Davidson, J. N., and Leslie, I., *Cancer Res.*, 1950, v10, 587.

14. Campbell, R. M., and Kosterlitz, H. W., *J. Physiol.*, 1947, v106, 12P.

15. Price, J. M., Miller, E. C., Miller, J. A., and Weber, G. M., *Cancer Res.*, 1950, v10, 18.

16. Price, J. M., and Laird, A. K., *Cancer Res.*, 1950, v10, 6[illegible]9.

17. Cohn, W. E., *Science*, 1949, v109, 377.

18. Hotchkiss, R. D., *J. Biol. Chem.*, 1948, v175, 315.

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Coccidioides immitis Spherule Antigen in a Complement Fixation Test For Experimental Coccidioidomycosis. (19440)

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Prior to 1937 few investigators were successful in demonstrating an immune response to *Coccidioides immitis* in patients with coccidioidal granuloma. Cooke(1) obtained a positive precipitin test with serum from a patient using antigens prepared from dried powdered cultures, and extracts of pus-containing spherules. Davis(2) obtained a positive complement fixation test with serum from a patient using an antigen prepared from a macerated mycelial culture. Chipman and Templeton(3), however, were the first to use successfully a broth filtrate of *Coccidioides immitis* as an antigen in a complement fixation test. This type of antigen has since proven effective and is now widely used. C. E. Smith and associates(4) have used the broth filtrate, coccidioidin, as antigen in a precipitin test and complement fixation test for diagnosis and prognosis in coccidioidomycosis.

In a preliminary report(5) it was shown that the spherules or tissue phase of *Coccidioides immitis* could be obtained in abundance by inoculation of the yolk sac of chick embryos with arthrospores of *Coccidioides immitis*. The use of the spherules as antigen in the complement fixation test is presented in this paper.

Methods. The yolk sacs of 10-day-old embryonated hens' eggs were inoculated with the arthrospores of *Coccidioides immitis* as described in a previous report(5). Up to 0.5 ml of egg yolk from the initially inoculated embryos was passed to the yolk sac of a second series of 10-day-old embryos. After 9 days incubation the yolk of the second transfer eggs was harvested and treated with 4 parts of 10% NaCl, and allowed to stand overnight in the refrigerator. The suspension of yolk and spherules was centrifuged at 3000

r.p.m. for ½ hour. The fat which collected at the surface during centrifugation was decanted along with the soluble egg protein in the supernate. The sediment containing the spherule material was washed several times with physiological saline and finally suspended in veronal buffer solution, pH 7.3-7.4. A 1:50 concentration of spherules by volume was obtained from 10 eggs. Equal aliquots of spherule suspensions were treated with a) 5% formalin for 12 hours, and b) heat-killed in a 60°C water bath for 30 minutes. Excess formalin in the spherule material was neutralized with sodium metabisulfite as recommended by Cox.* Before use in the complement fixation test the antigens were diluted and ground for ½ hour in a small Ten Broeck glass tissue grinder. *Coccidioidin. Coccidioides immitis,* strain 2023, was grown in a synthetic medium(6) for 4 months, the fluid content filtered through a Seitz filter and the filtrate stored immediately at —20°C without preservative. This filtrate, a clear amber colored fluid, was found to be a suitable antigen for the complement fixation test in an optimal dilution of 1:16. *Sera from infected animals.* Seven white rabbits, each weighing approximately 2 kg, were inoculated intravenously with 1 ml of a 1:1000 concentration (by volume) of Coccidioides arthrospores. Two weeks later the rabbits were bled to death and their sera was stored in small amounts at —20°C without preservative. *Sera from hyperimmunized animals.* Six white rabbits each weighing approximately 2 kg, were inoculated intravenously with 1 ml of a 1:50 concentration of spherule antigen on 4 consecutive weeks and were bled to death 7 days after the last inoculation. The sera were stored in small amounts at —20°C without preservative. At no time during the vaccination period did the animals show any reaction to the injected material. *Complement fixation test.* Three 50% units of guinea pig complement(7) were used in the test. All reagents were diluted in veronal buffer, pH 7.3-7.4, and used in a volume of 0.3 ml. A dilution of 1:3 serum, inactivated at 60°C for 30 minutes, served as unity for subsequent 2-fold, serial dilutions. After 16-18 hours refrigeration at 3-6°C, 0.6 ml of optimally sensitized sheep cells were added and the tubes placed in a water bath at 37°C for 30 minutes. All tubes were centrifuged at 2500 r.p.m. and the degree of hemolysis was compared with color standards prepared to represent 0, 25, 50, 75, and 100% hemolysis interpreted as 4+, 3+, 2+, 1+ and no fixation. Tubes showing 50% hemolysis or less were considered positive. *Absorption of infected and hyperimmune sera.* The absorption technic outlined by Krumweide *et al.*(8) was used to absorb non-inactivated sera from infected and hyperimmunized rabbits. The egg yolk antigen used for absorption was prepared by suspending the yolk from one 18-day-old incubated egg in distilled water. Three ml aliquots of a 1:3 dilution of serum from a hyperimmunized rabbit and an infected rabbit were added to a) the washed sediment of 4 ml of centrifuged egg yolk antigen, and b) the centrifuged sediment of 5 ml of stock spherule vaccine. The sera were placed in a water bath at 37°C for 5 hours and then refrigerated for 15 hours. The tubes were shaken often during the incubation periods. It was necessary to repeat the test twice to obtain complete absorption of the sera.

Results. The antigenic titer of the heat killed spherule antigen was taken as that dilution of antigen beyond which further increase failed to enhance complement fixation. The antigen, despite its concentrated appearance, was not anticomplementary either undiluted or diluted 1:2. The optimal antigen dilution for testing in rabbit sera was 1:4. Formalized antigen failed to fix complement in these experiments. The results of complement fixation tests with sera from 7 rabbits inoculated with *Coccidioides* arthrospores using coccidioidin, heated spherule antigen and antigen prepared from egg yolk are presented in Table I. The titers obtained with the spherule antigen were slightly higher in some instances than those to coccioidin. The egg yolk antigen failed to fix complement in these sera, while all three antigens failed to react in the sera from 6 normal rabbits tested independently.

* Personal communication.

TABLE I. Reactions in Complement Fixation Test Employing Heated Spherule Antigen, Coccidioidin, and an Egg Yolk Antigen and Serum from Rabbits Infected with *Coccidioides immitis*.

Antigens	Rabbit	Serum dilutions 1-1	1-2	1-4	1-8	1-16	1-32	1-64	Controls Antigen	Serum
1	28	4+	4+	4+	2+	—	—	—	—	—
2		4+	4+	4+	4+	2+	—	—	—	
3		—	—	—	—	—	—	—	—	
1	23	4+	4+	4+	3+	—	—	—	—	—
2		4+	4+	4+	3+	—	—	—	—	
3		—	—	—	—	—	—	—	—	
1	27	4+	4+	4+	4+	4+	2+	—	—	—
2		4+	4+	4+	4+	4+	2+	—	—	
3		—	—	—	—	—	—	—	—	
1	11	4+	4+	4+	3+	—	—	—	—	—
2		4+	4+	4+	4+	3+	1+	—	—	
3		—	—	—	—	—	—	—	—	
1	12	4+	4+	4+	2+	—	—	—	—	—
2		4+	4+	4+	4+	4+	3+	2+	—	
3		—	—	—	—	—	—	—	—	
1	14	4+	4+	4+	3+	2+	—	—	—	—
2		4+	4+	4+	4+	3+	1+	—	—	
3		—	—	—	—	—	—	—	—	
1	31	4+	4+	4+	4+	2+	—	—	—	—
2		4+	4+	4+	4+	2+	1+	—	—	
3		—	—	—	—	—	—	—	—	
1	Normal*	—	—	—	—	—	—	—	—	—
2		—	—	—	—	—	—	—	—	
3		—	—	—	—	—	—	—	—	

* Represents the reaction of 6 rabbit sera tested independently.
1—Coccidioidin. 2—Spherule. 3—Egg yolk.

Hyperimmune sera. The sera from 6 rabbits immunized with heat killed spherule antigen gave high titers to both spherule antigen and egg yolk antigen revealing the complicating factor of a concurrent stimulation of egg yolk antibodies during the immunization with this antigen (Table II). In only a few instances was it possible to demonstrate a titer to coccidioidin and, when evident, always in a low titer.

Absorption studies. Egg yolk antigen completely removed egg yolk antibody in the serum from immunized rabbit No. 6 (Table II). After absorption, this serum showed a reduction in titer to 1:4 with spherule antigen while the titer to coccidioidin was unchanged. Absorption of this serum with spherule antigen completely removed antibodies to spherule antigen, egg yolk and coccidioidin. Absorption of a serum from an infected rabbit with egg yolk antigen and spherule antigen failed to lower the titer to spherule antigen or coccidioidin.

Discussion. The spherule antigen prepared from the yolk of embryonated eggs inoculated with the arthrospores of *Coccidioides immitis* appears to have the ability to fix complement in the sera of rabbits infected with *Coccidioides immitis* and in the sera of rabbits hyperimmunized with the spherule antigen. The spherule antigen is also capable of stimulating in low titer, antibodies to coccidioidin, the broth filtrate, which is used in the standard complement fixation test for coccidioidomycosis. A preliminary testing in human serum with both antigens was made. The spherule antigen failed to react in the serum of a patient with coccidioidal granuloma showing a titer of 1:32 to coccidioidin.

Summary. 1. A method is described for the preparation of a serologically active antigen from egg yolk material containing spherules. 2. "Spherule antigen" used in a complement fixation test reacted with sera from animals infected with *Coccidioides* and animals hyperimmunized with the antigen. 3. The antigen

TABLE II. Reaction in Complement Fixation Test Employing Heated Spherule Antigen, Coccidioidin, and an Egg Yolk Antigen and the Sera from Rabbits Immunized Intravenously with Spherule Vaccine.

Antigens	Rabbit	Serum dilutions 1-1	1-2	1-4	1-8	1-16	1-32	1-64	1-128	Controls Antigen	Serum
1	5	—	—	—	—	—	—	—	—	—	—
2		3+	3+	3+	3+	3+	3+	1+	—	—	
3		2+	3+	3+	2+	—	—	—	—	—	
1	1	—	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	2+	—	—	—	—	—	
3		4+	3+	-+	—	—	—	—	—	—	
1	6	4+	2+	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	3+	1+	—	—	—	
3		4+	4+	4+	2+	2+	1+	—	—	—	
1	2*	2+	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	3+	2+	—	—	—	
1	4*	1+	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	4+	3+	2+	—	—	
1	3	1+	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	3+	3+	—	—	—	
3		4+	4+	4+	4+	2+	—	—	—	—	

4—Complete fixation (no hemolysis); –, no fixation (complete hemolysis).
* Sera from these rabbits were not tested with egg yolk antigen.
1—Coccidioidin. 2—Spherule. 3—Egg yolk.

was found to stimulate low titers to coccidioidin in hyperimmunized animals. 4. The "Spherule antigen" completely absorbed antibodies to the homologous antigen and coccidioidin from the serum of a hyperimmunized rabbit, but failed to absorb antibodies from the serum of a rabbit infected with *Coccidioides immitis*.

1. Cooke, J. V., *Arch. Int. Med.*, 1915, v15, 479.
2. Davis, D. S., *Arch. Dermat. and Syph.*, 1924, v9, 577.
3. Chipman, E. D., and Templeton, A. J., *Arch. Dermat. and Syph.*, 1930, v21, 259.
4. Smith, C. E., Saito, M. T., Beard, R. R., Kepp, R. M., Clark, R. W., and Eddie, B. U., *Am. J. Hyg.*, 1950, v52, 1.
5. Vogel, R. A., and Conant, N. F., *J. Bact.*, 1952, in press.
6. Smith, C. E., Whiting, E. G., Baker, R. R., and Saito, M. T., *Rev. Tuberc.*, 1948, v57, 330.
7. Kent, J. F., and Rein, C. R., *Science*, 1948, v103, 598.
8. Krumweide, C., Cooper, G., and Provost, D. J., *J. Immunol.*, 1925, v10, 55.

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Identification of Ferritin in Blood of Dogs Subjected to Radiation from an Atomic Detonation.* (19441)

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Direct observations on the mesoappendix capillary bed in the roentgen ray irradiated rat demonstrated that a pronounced peripheral vasodepression occurred during the first post-irradiation week. Further investigation showed that this effect was not due to a direct effect of the radiation on the capillaries themselves but was the result of the appearance of a vasodepressor agent in the blood. It was shown that this vasodepressor was

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