explained as a deficiency of prothrombin itself or of any of the classical clotting factors. Rather it would seem that the newborn infant is unable to convert prothrombin to thrombin with the same speed and efficiency as the normal adult. This view would also explain the discrepancy observed in studies on such subjects in the past when their blood has been examined simultaneously by the one stage and two stage technics. Our experiments indicate that a factor is provided by normal plasma, aged plasma or serum that considerably enhances the conversion of prothrombin. Recently reported studies, as previously mentioned, have shown that one or more accelerator factors are involved in the normal blood clotting mechanism. At least one of these factors has been shown to be present in serum. It is our belief that deficiency of such a factor contributes to the clotting abnormalities of the newborn, heretofore attributed solely to a lack of prothrombin. Further investigation of this basic problem to confirm and amplify these findings is clearly required.

Two recent papers[15,18] demonstrate that a diminution of such a factor accounts for certain clotting changes observed in dicoumarolized subjects. The observation that dicoumarolized plasma exerts little effect upon the plasma of deficient infants suggests that the changes in both plasmas may be of similar nature.

Summary. 1. Evidence is presented indicating that the prolonged prothrombin times observed in the plasmas of newborn infants, studied by the one stage technic, cannot be adequately explained as a simple deficiency of prothrombin.

2. Although there may be some diminution of prothrombin, it appears that the plasma of the newborn is deficient in a factor accelerating conversion of prothrombin to thrombin. The deficiency of this factor is remedied by the addition of small amounts of normal plasma, stored plasma or serum.

18 Owen, C. A., and Bollman, J. L., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 231.

16878

Agglutination of Sheep's Erythrocytes Sensitized with Histoplasmin.

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The need of sensitive and specific serologic tests in fungus diseases has lately arisen in cases with skin reactions to histoplasmin. Several publications[1-3] describing complement fixation tests have appeared. Recently Saslaw and Campbell[4] have reported agglutination tests using collodion particles sensitized with histoplasmin. The preparation of collodion particles, however, is very difficult. Middlebrook and Dubos[5] have reported a similar agglutination test for tuberculosis in which sheep's erythrocytes were sensitized with a specific substance extracted from tubercle bacilli.

The present report describes an agglutination test using histoplasmin-sensitized sheep's erythrocytes as antigen and sera from rabbits immunized with *Histoplasma capsulatum.*

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1 Tennenberg, D. J., and Howell, A., *Pub. Health Rep.*, 1948, **63**, 163.

2 Salvin, S. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 342.

3 Saslaw, S., and Campbell, C. C., *J. Lab. and Clin. Med.*, 1948, **33**, 811.

4 Saslaw, S., and Campbell, C. C., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 559.

5 Middlebrook, G., and Dubos, R. J., *J. Exp. Med.*, 1948, **88**, 521.

TABLE I.
Determination of the Optimal Dilution of Histoplasmin (Lot H-40) for Hemagglutination. Serum Prepared in Rabbits Against the G-5 Strain of *H. capsulatum*.

Dil. of histoplasmin before mixture with red cells	Immune serum dilutions										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
1:1	—	—	—	—	—	—	—	—	—	—	—
1:2*	+	+	+	+	+	+	+	+	±	±	—
1:3	+	+	+	+	+	+	+	±	±	±	—
1:4	+	+	+	+	+	+	+	±	±	—	—
1:6	+	+	+	+	+	+	+	±	—	—	—
1:8	+	+	+	+	+	+	±	—	—	—	—
1:12	±	+	+	+	+	+	±	—	—	—	—

Dil. of histoplasmin before mixture with red cells	Controls. Immune serum 1:10	Normal serum 1:10	Normal serum 1:20	Normal serum 1:40	Saline
1:1		—	—	—	—
1:2		±	±	—	—
1:3		±	—	—	—
1:4		±	—	—	—
1:6		±	—	—	—
1:8		—	—	—	—
1:12		—	—	—	—
Normal red cells	—				

* Optimal dilution.

Methods. Histoplasmin lot H-40† was used in this study. The red cell suspension was prepared according to the method of Middlebrook and Dubos.[5] A 0.25% suspension, however, was found to give a more sensitive test and was used instead of the 0.5% suggested in the original description.

The sera from rabbits‡ immunized with 2 strains of *Histoplasma capsulatum* (G-5 and G-6, Army Medical Center, Washington, D. C.) were used. As controls, sera from 6 normal rabbits and from 2 rabbits infected with *Sporotrichum Schenckii* (Duke strain 2079) and finally the serum from a rabbit immunized with *Candida albicans* (Duke strain) were used.

The sensitized cells-serum mixture was incubated according to the method of Middlebrook and Dubos.[5]

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‡ Generously supplied by Miss Charlotte C. Campbell, Department of Bacteriology, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D.C.

The tests were read by the pattern formed by the sedimented red cells rather than by the size of the agglutinated particles, as this was found to give more accurate readings. A positive reaction (+), as described by Salk[6] in hemagglutination tests for influenza virus, showed a thin, brownish-yellow, adherent film covering the hemispherical bottom of the test tube. In a negative reaction (—), the cells rolled to the bottom and settled as a sharply demarcated red disc which was much less adherent to the wall of the tube and moved when the tube was tilted. Reactions intermediate between the homogeneous positive film and the negative disc were characteristically ring-like in form and somewhat larger than the disc in negative tubes (±). Since reactions of this degree appeared of doubtful significance they have been regarded tentatively as negative. Serum titer was defined as the highest dilution yielding a positive (+) reaction.

Results. The optimal dilution of histoplasmin lot H-40 was found to be a 1:2 dilution (Table I).

Undiluted histoplasmin hemolysed the

[6] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

TABLE II.
Results of Hemagglutination Tests with Histoplasmin (Lot H-40).

Sera of rabbits immunized with:	Serum dilution										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
H. capsulatum, G-5	+	+	+	+	+	+	+	+	±	±	—
" " G-6	+	+	+	+	+	+	±	—	—	—	—
C. albicans, M-1*	±	±	±	—	—	—	—	—	—		
S. schenckii, S-7†	±	±	±	—	—	—	—	—	—		
" " S-8†	±	±	±	—	—	—	—	—	—		
6 normal rabbits	—	—	—	—	—	—	—	—	—		

* Agglutination titer with *C. albicans* cells 1:2560.
† Agglutination titer with *S. schenckii* cells 1:2560.

sheep's red cells and therefore could not be used.

As shown in Table II the sera from 2 rabbits immunized with *H. capsulatum* gave titers of 1:1280 and 1:320, respectively. Sera from normal rabbits gave negative results. Sera from 2 rabbits infected with *Sporotrichum Schenckii* and from one rabbit immunized with *Candida albicans* yielded no positive reactions. These sera were shown, however, to contain antibodies against their homologous organisms by standard agglutination tests. Serum from one human case of histoplasmosis showed no antibodies.§

It would seem, therefore, from these preliminary results that this method might be useful in serologic tests for histoplasmosis.

Summary. An hemagglutination test is described using sheep's erythrocytes sensitized with histoplasmin and sera from rabbits immunized with *H. capsulatum*. Sera from normal rabbits gave negative results and no cross-reactions were found with sera containing antibodies against *S. Schenckii* and *C. albicans*.

§ Repeated complement fixation tests of this serum, done by Miss Charlotte C. Campbell, have been similarly negative.

16879

Histological Manifestations of a Magnesium Deficiency in the Rat and Rabbit.*

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According to Tufts and Greenberg[1] two phases are observed in magnesium deficiency in rats. The first is marked by vasodilatation, hyperemia, and hyperexcitability. The second phase is characterized by the development of nutritive failure, cachexia, and kidney damage. Greenberg, Lucia and Tufts[2] showed that prolonged deprivation of magnesium eventually produces degeneration in the kidneys, histologically manifested by degenerative changes in the tubules and progressive calcification in the cortico-medullary zone, and later, in the cortex. Renal damage was also observed in calves maintained on a diet

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1 Tufts, E. V., and Greenberg, D. M., *J. Biol. Chem.*, 1938, **122**, 693.

2 Greenberg, D. M., Lucia, S. P., and Tufts, E. V., *Am. J. Physiol.*, 1938, **121**, 424.