reached, by extrapolation of the regression line. (2) The dose response assay provides an indicator of titer at a single virus dilution. (3) The character of the dose response curve provides a check on the normality of mouse response.

As an indicator of infectivity for Friend Leukemia Virus infection, thrombocytopenia offers several definite advantages over splenomegaly. Spleen weight can vary widely with changes in the physiologic status of the animal. Starvation, infection and stress may produce significant changes in spleen weight.

It has been recently demonstrated that many strains of mice relatively resistant to the Friend virus on the basis of splenomegaly and the subsequent development of a leukemic state will nevertheless show significant thrombocytopenia after infection with the Friend virus(3). Therefore, the thrombocytopenia is probably a more sensitive indicator of infection than splenomegaly. Finally, using thrombocytopenia the virus titer can be obtained without killing the mouse.

Summary. The Friend Leukemia Virus causes significant thrombocytopenia within 48 hours after infection in Swiss Webster female mice. This thrombocytopenia has been demonstrated to be dependent upon the dilution of virus inoculated. A linear relationship exists between dose of virus and degree of thrombocytopenia. A dose-response curve using thrombocytopenia can be used to assay the Friend Leukemia Virus.

1. Nelson, E., Bierman, H., J.A.M.A., 1964, v190, 99.
2. Charkes, N. D., Ann. Int. Med., 1961, v54, 745.
3. Hudson, J. B., Weinstein, L., Chang, T. W., J. Pediat., 1956, v48, 48.
4. Ferguson, A. W., Pediatrics, 1960, v25, 400.
5. Kolars, C. P., Spink, W. W., J.A.M.A., 1958, v168, 2213.
6. Woodward, T. E., Ann. Int. Med., 1943, v19, 799.
7. Dennis, L. H., Brodsky, I., J. Nat. Cancer Inst., in press.
8. deHarven, E., Friend, C., J. Biophys. and Biochem. Cytol., 1960, v7, 747.
9. Dalton, A. J., Law, L. W., Moloney, J. B., Manaker, R. P., 1961, v27, 747.
10. Rowe, W. P., Brodsky, I., J. Nat. Cancer Inst., 1959, v23, 139.
11. Brecher, G., Cronkite, E. P., J. Appl. Physiol., 1950, v3, 365.
12. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture. Biology, 4th ed., Collegiate Press, Inc., Ames, Iowa, 1946, p485.
13. Reed, L. J., Muench, A., Am. J. Hyg., 1938, v27, 493.

Received September 9, 1965. P.S.E.B.M., 1965, v120.

Immunology and Serology of *Anaplasma marginale*. II. Nature of the CF Antigen.* (30625)

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A previous report(1) described the purification of *Anaplasma* complement-fixing (CF) antigen. The antigen was produced from the sonicate of *Anaplasma*-infected bovine red blood cells by a process of differential centrifugation followed by treatment with an ion exchange resin. The antigen possessed high CF titer, low anticomplementary activity and did not contain the color due to hemoglobin which interferes with interpretation of hemolysis in the CF test. Preliminary chemical studies indicated that the antigen was lipoprotein in nature. Since the CF antigen is employed routinely for diagnosis of anaplasmosis(2), studies were conducted to determine its nature.

* This investigation was supported in part by USPHS Research Grant AI-02250 and Training Grant 2E-184, from Nat. Inst. of Allergy and Infect. Dis.

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Materials and methods. CF antigen was prepared from the sonicate of red blood cells infected with *Anaplasma marginale* as previously described(1).

Filtration. Antigen was sedimented at 105,000 × *g*, resuspended in 50 volumes of phosphate-buffered saline, pH 7, and passed through a microfiber glass prefilter (Millipore). Subsequent filtrations were performed using 5000, 3000, 1200, 650 and 450 mμ pore sizes (Millipore). After each filtration a 5-ml sample of the filtrate was retained while the remainder was passed through the next filter in the sequence. Residues were washed once with phosphate-buffered saline, pH 7, after each filtration was completed. Filtrates were centrifuged for 90 minutes at 105,000 × *g*, and the sediments were resuspended in 5 ml of veronal-buffered saline, pH 7.2 (CF buffer) and titrated for CF antigenicity(3).

Antigenicity and Anaplasma *body count.* To determine the relationship of CF antigenicity to the number of erythrocytes containing *Anaplasma* bodies (AB), blood was obtained from normal and carrier calves and from calves in the acute stages of anaplasmosis with 6.5 to 90% of the erythrocytes containing AB. Packed cells from each animal in 200-ml volumes were used in preparing separate antigens by the method indicated. Aliquots of each preparation were titrated, and CF antigenicity was compared.

Microscopic examination. Smears of the undiluted antigens were prepared and stained with Giemsa stain. Uranium-shadowed preparations of the antigen were also examined by electron microscopy.

Biochemical characteristics. Tests for protein were performed using the xanthoproteic and Millon's tests; the anthrone and Molisch tests were used for detection of carbohydrate. Tests for cystine were made by heating a mixture of lead acetate with a sample of antigen dissolved in 1 N NaOH(4). The presence of sterol was determined by the Salkowski and Liebermann-Burchard tests. Lipid analysis was made using the thin-layer chromatography technique(5) to detect classes of lipids. Quantitative protein determinations were also made using the biuret test.

The antigen was exposed to trypsin, lipase, takadiastase, phospholipase A, DNAse and RNAse, to determine their effects upon the CF antigenic substance. Antigen was diluted 1:5 with CF buffer, distributed in 5-ml aliquots, and enzymes were added to give the final concentration indicated in Table III. The protocol describing the pH, activator and concentration of enzymes used in these experiments has been described(6). The mixtures of antigen and enzymes were incubated for 1 hour at 37°C and then 5 hours at 4°C. The treated antigen samples were sedimented by centrifuging for 20 minutes at 39,000 × *g* and washed 3 times with CF buffer by the same process. The washed sediments were reconstituted to 5 ml with CF buffer and titrated.

Effects of temperature. Antigen was incubated in a water-bath for 2 hours at 60°C. Additional samples remained at 25°C for 12 hours and others were frozen and thawed several times. Titrations were performed on all samples to determine the effects of these temperature treatments on the CF antigenic activity of the purified antigen.

Results. Filtration studies. Antigenic activity of serial filtrates of the purified *Anaplasma* CF antigens appears in Table I. These results indicated the antigenic substance to be of relatively large particle size. The antigenically-active material passed through filters with apparent pore diameters of 650 mμ and greater, but material passing through filters of 450 mμ failed to show antigenic activ-

TABLE I. CF Antigenic Activity of Filtrates* of CF Antigen Prepared from Sonicates of *Anaplasma*-Infected Erythrocytes.

Antigen (ml)	Unfiltered control	Pore size in mμ 5000	3000	1200	650	450
.012	—	—	—	—	—	—
.025	4	4	4	—	—	—
.050	4	4	4	3	2	—
.100	4	4	4	4	4	—
.150	4	4	4	4	4	—
.200	4	4	4	4	4	—
.250	4	4	4	4	4	—
.400	4	4	4	4	4	—
.500	4	4	4	4	4	—

* Passed through *Millipore* filters.

Negative (—) through 4 indicates degree of complement-fixation.

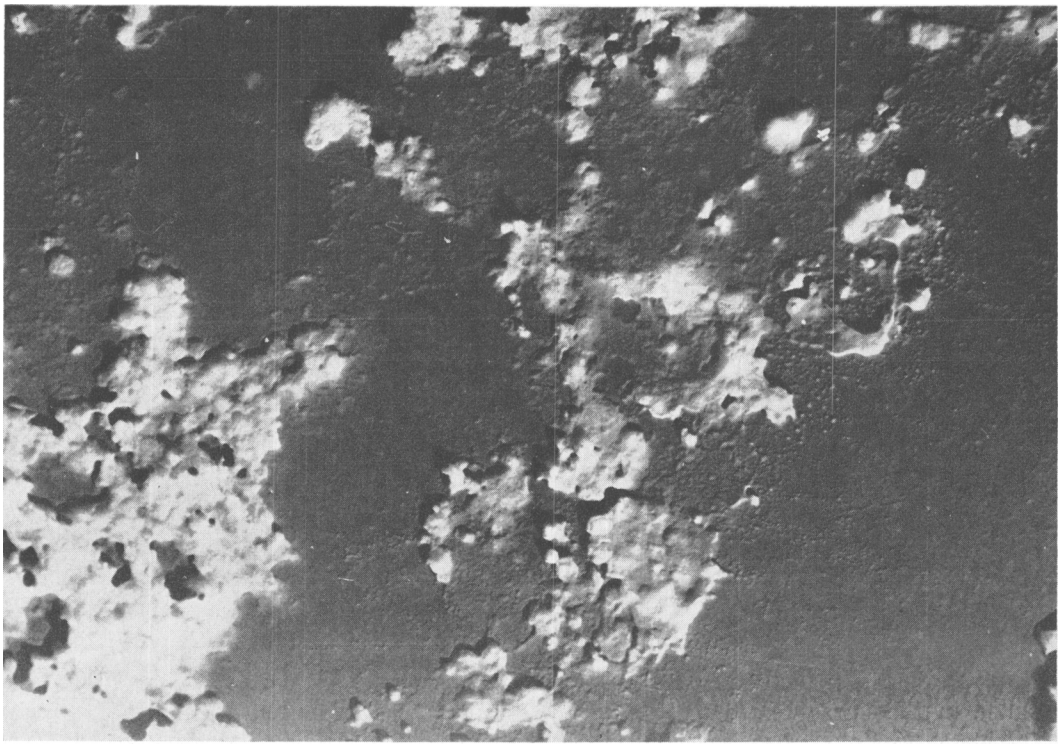

FIG. 1. Electron micrograph of *Anaplasma* CF antigen prepared from the sonicate of infected bovine erythrocytes. Uranium shadowed. 4950×.

ity. Initial filtrations resulted in more homogenous suspensions of the material, but as filters with progressively smaller pore diameters were used, the suspensions became clearer. The filtrate which was passed through the 450 mμ filter was clear and colorless.

Antigenicity and Anaplasma *body count.* Similar antigens were prepared from the sonicates of *Anaplasma*-infected erythrocytes obtained from carrier animals and animals in the acute stages of the disease with 6.5 to 90% of the erythrocytes containing AB. The CF antigenicity increased as the number of erythrocytes containing AB increased. Table II shows the relative mean CF titers of antigens prepared from equal quantities of packed *Anaplasma*-infected red blood cells in relation to the percentage of cells containing AB. In the course of this investigation similar observations, which support these results, were made after preparing numerous other experimental CF antigens.

Microscopic examination. Examination of antigens produced in this manner by electron microscopy and after staining with Giemsa revealed only amorphous stromatal material. Fig. 1 is an electron micrograph of an uranium-shadowed preparation of the antigen suspension. No AB were apparent.

Biochemical characteristics. A number of qualitative biochemical tests were made on antigens produced from sonicates of *Anaplasma*-infected red blood cells. The results of tests for protein, lipid and sterol were positive while no carbohydrate or cystine was detected. Protein concentration was estimated at 1.98%. Phosphatide and sterol were the only lipid classes detected by thin-layer chromatography.

The antigen was treated with various enzymes and titrated to determine the effects of enzymatic action on the antigenic titer. The results of these experiments appear in Table III. Only 2 of the enzymes had apparent destrucutive effects upon the CF activity of the antigen. The trypsin-treated sample showed almost a complete loss in antigenic activity, and a significant reduction in titer

TABLE II. Mean Relative CF Activity of Antigens Prepared from the Sonicates of Erythrocytes with Various AB Counts.

Antigen (ml)	% RBC containing AB: Normal (control)	Carrier*	6.5	36.5	45	65	75 & 90†
.012	—	—	—	—	—	—	4
.025	—	—	—	—	4	4	4
.050	—	—	—	1	4	4	4
.100	—	—	—	2	4	4	4
.150	—	—	—	3	4	4	4
.200	—	—	—	4	4	4	4
.250	—	2	1	4	4	4	4
.400	—	2	2	4	4	4	4
.500	—	3	4	4	4	4	4

* Less than 1% of erythrocytes containing AB.
† Pooled.
Negative (—) through 4 indicates degree of complement-fixation.

TABLE III. Effects of Enzymes on *Anaplasma* CF Antigen.

Antigen (ml)	Antiserum	Untreated antigen	Concentration of enzyme (%): Trypsin (.35)	Takadiastase (.75)	Phospholipase A (.10)	Lipase (.75)	DNAase (.50)	RNAase (.50)
.1	present	4	—	4	4	—	4	4
.1	absent	—	—	2	—	4	—	—

Negative (—) through 4 indicates degree of complement fixation.

resulted in the lipase-treated antigen. Considerable anticomplementary activity was exhibited by the samples treated with lipase and takadiastase, although samples exposed to phospholipase A and the nucleases showed a small degree of anticomplementary activity at lower antigen dilutions.

Effects of temperature. Samples of the antigen were treated at 60°C for 2 hours, at 25°C for 12 hours and repeatedly frozen and thawed. No changes in CF antigenic titer resulted from these treatments.

Discussion. A number of studies were made on the antigen prepared from sonicates of *Anaplasma*-infected erythrocytes in an effort to determine the nature of the CF antigen and its relationship to the AB. Filtration studies of the experimental antigen showed that the CF antigenic substance passed through filters with pore diameters of 650 mμ. However, no activity was found in filtrates which passed through filters having a pore size of 450 mμ. These results were rather surprising, since Allbritton and Parker(7) conducted filtration studies which showed that the infective agent passed through filters having average pore diameters of 300 mμ. The amount of material required to produce infection might, however, be much less than that required for detection by the CF test. The presence of a small number of elementary bodies might induce infection but it is not likely that a minute amount of material would possess sufficient antigenic activity to be detectable by the CF test. This is one possibility, since the size range of the infective agent was estimated to be between 220 and 300 mμ(7,8). Generally, it is recognized that the CF active substance in viruses is smaller than the infectious particle. However, in the present studies the CF antigenic substance appeared to be composed of relatively large conglomerates of amorphous material.

Another possibility is that the infectious material which passed through the pores of 350 mμ diameter(7) may not have possessed any CF antigenicity. There is support for this in the fact that suspensions of concentrated semi-purified AB possessed very low CF antigenic activity(1). The CF activity was present in a fraction composed of stromatal material and few AB.

The activity of antigens prepared from the sonicates of infected erythrocytes increased as the number of erythrocytes containing AB increased. Similar observations were reported

by Franklin *et al*(9). However, this should not necessarily imply that the AB, *per se*, constitutes the CF antigen. To the contrary, antigens prepared by this method contained little or no AB, as revealed by electron microscopy of suspensions of such preparations. Examination of these antigens again revealed only amorphous stromatal material. These results indicate that the CF antigens may reside either on the outer matrix of the AB, which is stripped away during the purification process, or elsewhere in or on the infected erythrocyte. Ristic and Kreier(10) have reported that a capillary agglutinating (CA) antigen, prepared from *Anaplasma*-infected erythrocytes(11) consists of structures resembling the subunits (initial bodies) of *Anaplasma* marginal inclusion bodies. They likewise reported an outer envelope covering the marginal bodies. It is this outer matrix which apparently constitutes the CF antigen previously purified(1). The individual subunits do not appear to possess appreciable CF antigenicity.

A previous report(1) including the results of studies on partial and total lipid extractions of the CF antigen suggested that the CF antigen was a lipoprotein. Results presented herein confirm the earlier observation.

Summary. A CF antigen for *Anaplasma marginale* was prepared by differential centrifugation of the sonic lysate of infected bovine erythrocytes. Studies were conducted to determine the chemical and physical nature of the antigen. Qualitative biochemical tests and enzyme sensitivity studies indicated the antigen to be lipoprotein in nature. Electron microscopic observations showed that the preparation was not composed of *Anaplasma* bodies but of amorphous membranous material which probably constitutes the outer coating of the AB. Filtration studies were conducted and observations made on the effect of temperature on the antigen.

1. Rogers, T. E., Hidalgo, R. J., Dimopoullos, G. T., J. Bacteriol., 1964, v88, 81.

2. Gates, D. W., Roby, T. O., Ann. N. Y. Acad. Sci., 1956, v64, 31.

3. U. S. Dept. of Agriculture, A Manual for Conducting the CF Test for Anaplasmosis, Laboratory Services, Animal Disease Eradication Division in Cooperation with Animal Disease and Parasite Research Division, Washington, D. C., Aug. 1958.

4. Harrow, B., Borek, E., Mazur, A., Stone, G. C. H., Wagreich, H., Laboratory Manual of Biochemistry, W. B. Saunders Co., Philadelphia, 4th ed., 1955.

5. Mangold, H. K., J. Am. Oil Chemists Soc., 1961, v38, 708.

6. Long, C., Biochemist's Handbook, D. Van Nostrand Co., Inc., Princeton, N. J., 1961.

7. Allbritton, A. R., Parker, L. T., Am. J. Vet. Res., 1962, v23, 809.

8. Ristic, M., ibid., 1960, v21, 890.

9. Franklin, T. E., Heck, F. C., Huff, J. W., ibid., 1963, v24, 483.

10. Ristic, M., Kreier, J. P., ibid., 1963, v24, 985.

11. Ristic, M., J.A.V.M.A., 1962, v141, 588.

Received September 9, 1965. P.S.E.B.M., 1965, v120.

Renal Vascular Responses to Drugs in Experimental Nephrosis in Rats. (30626)

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Animal models of nephrosis are characterized by morphologic abnormalities of glomerular epithelial cell foot processes and basement membranes as well as edema, proteinuria, hypoproteinemia and hyperlipemia(1-3). These symptoms are also typical of the human disease(2-5). Experimental models can be divided into two major groups: a) those produced by various antibodies to kidney proteins and b) those induced by chemical agents, *e.g.*, puromycin aminonucleoside (4,5). In view of the marked histological and