lated during 24 hours of incubation at 37.5°C. Seven strains of staphylococci (4 aureus, 2 albus and 1 citrus) were then used to study the effect of heparin on the coagulation of plasma. Each of the series of 3 tubes containing the different dilutions of heparin was coagulated by 4 of these 7 strains of staphylococci. Physiological saline in quantities equal to that of heparin was added to a series of tubes of the citrated plasma for the controls. They were inoculated simultaneously with the different strains of staphylococci. Each of the strains of staphylococci that coagulated the heparinized citrated plasma also coagulated the control medium. This citrated plasma medium, containing both heparin and saline, did not coagulate during the time of incubation when staphylococci were not added. When citrated blood was substituted for the plasma, coagulation occurred in a similar manner with the four of the seven strains of staphylococci.

It is evident, therefore, from these observations that heparin, even in a 25.0% concentration, does not inhibit the coagulation of citrated human and rabbit plasma and also blood by the strains of staphylococci containing the so-called coagulase factor.

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Bovine Albumin as an Antigen.*

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The possible use of bovine albumin in man[1, 2, 3] makes the behavior of bovine albumin as an antigen of some interest. Previous reports[4, 5, 6] indicate that bovine albumin behaves as a potent antigen

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1 Janeway, C. A., and Beeson, P. B., *J. Clin. Invest.*, 1941, **20**, 435.

2 Davis, H. H., Eaton, A. G., and Williamson, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 96.

3 Taylor, H. L., Keys, A., and Savage, G., PROC. SOC. EXP. BIOL. AND MED., 1942, in press.

4 Leblanc, A., *La Cellule*, 1901, **18**, 335.

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TABLE I.
Anaphylactic Response of Sensitized Guinea Pigs to Single Intracardial Injections of Bovine Albumin and Globulin.

	Albumin				Globulin			
Days after inj.	7	14	18	21	7	14	18	21
No. of animals tested	3	3	3	7	3	2	4	2
No. of animals showing symptoms	0	0	3	7	0	2	4	2
No. of animals dying from anaphylaxis	0	0	3	4	0	2	4	1

in rabbits and guinea pigs. This paper is to report some studies on the antigenic behavior of bovine albumin in rabbits, guinea pigs, and in man.

Bovine albumin was prepared as described elsewhere[3] by fractionation of plasma with ammonium sulfate. Electrophoretic analysis was carried out by the methods of Tiselius[7] and Longsworth.[8]

Three rabbits were given a course of 6 injections of bovine albumin over a period of 4 weeks. Serum from blood drawn at the end of this period gave a titer as high as 24,000. Sixteen guinea pigs were given a sensitizing dose of 50 mg of albumin per kg and were tested with a shock dose of the same size administered intracardially. Eleven guinea pigs were similarly treated with globulin. All animals which died after the shock dose were posted. The results are given in Table I.

The bovine albumin had a longer sensitization period than globulin. A similar difference has been reported between horse albumin and globulin.[9]

The following case history is definite evidence that bovine albumin may act as an antigen in man. Patient N. R. was given 10 cc of 6% albumin intravenously on November 11, 1939. The skin test to albumin, which had been previously negative, was positive on December 18, 1939, at which time 3 cc of 8.8% albumin solution were administered intravenously over a 5-minute period. At the end of this time the pulse was weak, the respiration was shallow, and cynanosis developed. The injection was stopped. Respiration ceased momentarily but the patient responded to intravenous adrenaline and artificial respiration. After 5 minutes he recovered completely and had no complaints.

A positive skin test does not necessarily indicate that the patient will have anaphylactic symptoms. Four patients with mildly positive skin reactions were given 10 cc of albumin without effect. One

7 Tiselius, A., *Faraday Soc.*, 1937, **33**, 524.

8 Longsworth, L. G., *J. Am. Chem. Soc.*, 1939, **61**, 529.

9 Doerr, R., and Berger, W., *Z. Hyg.*, 1922, **96**, 191.

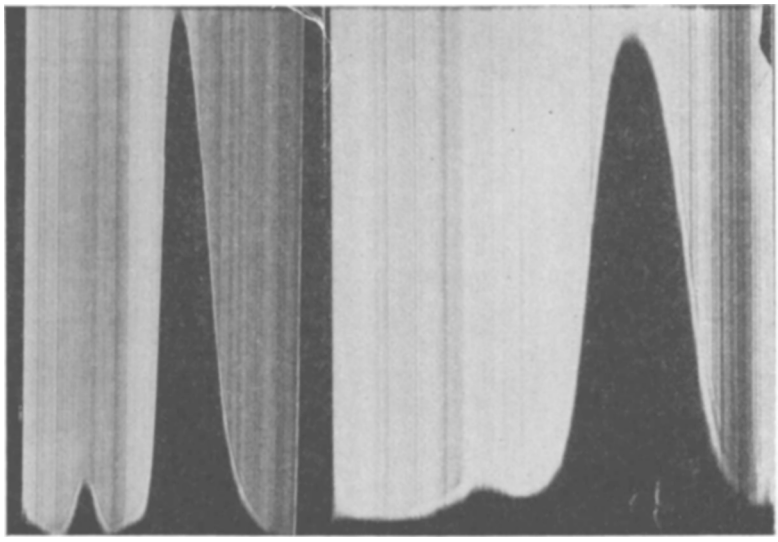

FIG. 1.
Electrophoretic patterns (descending boundary) of bovine albumin in phosphate buffer ionic strength = 0.05 pH = 7.4; left, prepared with ammonium sulfate; right, prepared with methyl alcohol.

patient with a 2+ skin reaction was given 1 cc intravenously followed by the immediate development of urticaria.

Discussion. Representative electrophoretic patterns of the albumin used in this work appear in Fig. 1. A pattern of albumin prepared with methyl alcohol recommended by us[3] for intravenous use is also included. The antigenic properties of this material have not been studied in detail but it can be expected to give similar results since it has approximately the same electrophoretic pattern and it has been shown[10] that the immunological properties of alcohol precipitated serum protein fractions do not differ from $(NH_4)_2SO_4$ treated fractions. Both preparations show 5-7% of a component moving slower than the albumin. Reasons for believing that this slow-moving component does not consist entirely of albumins will be given elsewhere. Some of the anaphylactic properties of these preparations may be due to residual globulin. The work of Svensson,[11] Cohn, *et al.*,[12] and our own experience indicates that it is not possible to prepare albumin free of the slower moving component by the simple fractionation procedures which are easily adapted to

10 Chu, C. T., and Chan, C. G., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 323.

11 Svensson, H., *J. Biol. Chem.*, 1941, **139**, 805.

12 Cohn, E. J., McMeekin, T. L., Oncley, J. L., Newell, J. M., and Hughes, W. L., *J. Am. Chem. Soc.*, 1940, **62**, 3386.

large-scale production. It is obvious that these procedures are inadequate to prepare non-antigenic albumin. Complete elimination of the slower-moving component may produce a preparation which does not have such striking antigenic properties.

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Intravenous Administration of Bovine Plasma Albumin.*

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Kremen, Taylor, and Hall[1] found that the globulin fraction of bovine plasma produced far more skin reactions than the albumin fraction. Whole bovine plasma produces an alarming number of untoward reactions when administered intravenously to man.[2] Satisfactory preliminary results with the intravenous administration of the albumin fraction of bovine plasma prepared by ammonium sulphate fractionation have been reported recently.[3] The present article is a report on the methods of preparation and results of the intravenous administration of plasma albumin.

Bovine albumin was prepared by fractionation from sterile plasma with (a) ammonium sulfate and (b) methyl alcohol.

Solid ammonium sulfate was dialyzed into sterile plasma through a cellophane membrane at +4 C; 350 g ammonium sulfate were used for each liter of plasma. The albumin was precipitated from the filtrate of the plasma by saturation with ammonium sulfate. The final precipitate was dissolved in distilled water and was dialyzed in collodion bags against a phosphate buffer at pH 7.4, ionic strength = 0.155. The albumin solution was Seitz-filtered, tested for sterility and stored in sealed vials at 4°C until used. Contamination was kept at a minimum by clean technic and main-

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2 Wangensteen, O. H., Hall, H., Kremen, A. J., and Stevens, B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 616.

3 Davis, H. H., Eaton, A. G., and Williamson, J., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 96.