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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1 Kremen, A. J., Taylor, H. L., and Hall, H., PROC. SOC. EXP. BIOL. AND MED., 1940, **42**, 532.

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.

tenance of temperature at 4°C, but complete sterility during preparation was not achieved except in the final solution.

Pyrogenic response occurred rather frequently when these ammonium sulfate preparations were administered. For comparison, fractionations were made with methyl alcohol and a rigorously controlled aseptic technic. The bacteriological details will be presented elsewhere.[4] All procedures were carried out in a sterile atmosphere (ultraviolet lamps and air filters). At each stage of preparation samples were taken and cultured at 5°, 25°, and 37°C, both aerobically and anaerobically. If contamination appeared at any stage, the lot was discarded.

Sterile bovine plasma was precipitated in liter centrifuge bottles at −17°C by adjusting the methyl alcohol concentration to 40%. After centrifugation and aseptic transfer of the supernatant liquid, the albumin was precipitated by increasing the methyl alcohol concentration to 70%. This precipitate was washed with 95% and 100% methyl alcohol and 3 times with absolute ethyl ether. It was then dried *in vacuo* and stored as a dry powder until used. For administration the powder was dissolved in sterile pyrogen-free normal saline solution and the solution finally filtered through a Berkefeld N filter.

Large quantities of albumin prepared with ammonium sulfate have been given to dogs, rabbits, and guinea pigs. Two dogs were given one gram of albumin per kilo intravenously without any noticeable effects. Three dogs under pentobarbital sodium anesthesia, with the carotid artery cannulated for blood pressure measurements, were given 0.5 g of albumin per kilo in solutions whose concentrations ranged from 3 to 12% of albumin. No significant change of blood pressure was noted. The blood pressure of one dog was reduced to the shock level of 55 mm Hg by hemorrhage. The blood pressure was restored to its original level by intravenous administration of 100 cc of 10% albumin solution.

The results in man of the intravenous administration of albumin prepared with ammonium sulfate are summarized in Table 1-A. Eight preparations were tested. Four preparations tested with 18 individuals gave no reactions; 4 other preparations tested with 8 individuals gave reactions in 7 cases.

The results of the administration of the methyl alcohol albumin are given in Table I-B. Five preparations tested in 14 patients gave only 3 reactions; of these, 2 reactions can be attributed to chill-producing substances carried over from bovine plasma.

[4] Savage, G. M., Taylor, H. L., and Keys, A., in press.

TABLE I.
Summary of Results Obtained with Intravenous Injection of Bovine Albumin.

A Prepared by ammonium sulphate fractionation				B Prepared by methyl alcohol fractionation			
Lot No.	No. patients tested	G albumin adm. per patient	No. of reactions	Lot No.	No. patients tested	G albumin adm. per patient	No. of reactions
12	3	0.7	0	$4C_6$	4	3.0	1
15	3	0.5	0	$4C_6$	1	3.0	urticaria
17	9	0.7	0	$4B_5$	4	3.0	0
22	3	0.5	2	3	3	3.5	1
27	3	9.0	0	$4B_4$	2	3.6	0
29	2	0.6	2				
34	2	0.6	2				
41	1	6.0	1				

TABLE II.
Types of Reactions Encountered on Administration of Bovine Albumin.

Patient	Lot No.	Prep. type	G albumin adm. per patient	Immediate reactions 0-30 min	Delayed reactions			
					Max. temp.	Nausea vomiting	Diarrhea	Chill
T.C.	22	$(NH_4)_2SO_4$	0.5	none	100.8	none	none	mild
C.L.	22	”	0.5	”	100.8	”	”	”
N.W.	29	”	0.6	”	103	”	+	moderate
N.P.	29	”	0.6	”	normal	+	+	mild
E.P.	34	”	0.6	”	102	none	none	moderate
W.B.	34	”	0.6	”	104	”	”	severe
N.O.	41	”	6.0	”	—	”	”	moderate
E.C.	3	Alcohol	3.5	”	102	”	”	mild
J.Lab.	$4C_6$	”	3.0	”	100.8	”	”	”
A.F.*	$4C_6$	”	0.1	urticaria	none	”	”	none

*Positive skin test 2+.

No significant changes in blood pressure were found during or following the administration of bovine albumin in patients with relatively normal blood pressures at the start. Albumin has been administered in concentrations as high as 15% without influencing blood pressure or pulse rate.

The types of reactions are given in Table II. Severe reactions resulted from the intravenous administration of small amounts of albumin prepared by ammonium sulfate precipitation without proper bacteriological control.

Discussion. The reactions obtained with the intravenous administration of preparations 22, 29, 34, and 41 are attributed in large measure to contamination during preparation and failure to eliminate the resulting pyrogens. The most common contaminants during the winter were found to be cryophyllic organisms present on the cattle. These organisms were apparently brought into the laboratory on the bleeding equipment and were found in the ammonium sulfate preparation at a later stage. We have not found it possible consistently to maintain sterile solutions throughout the fractionation of beef plasma with ammonium sulfate.

The advantage of the use of methyl alcohol or other bacteriostatic organic solvents in the preparation of bovine albumin for intravenous use is obvious. Complete control of sterility during the preparation is essential. The ordinary procedures of fractionation do not eliminate pyrogens once they are formed.

The work of Kremen, *et al.*,[5] suggests that chill-producing substances other than those of bacterial origin may be present in bovine blood. The appearance of two reactions of the chill type in the alcohol preparations which were not contaminated at any time suggests that a single fractionation with methyl alcohol may not completely eliminate the chill-producing substances.

Conclusions. 1. Methyl alcohol is preferable to ammonium sulfate for the preparation of bovine albumin for intravenous use. 2. Bovine albumin can be prepared which gives a low incidence of reactions when administered intravenously to man.

[5] Kremen, A. J., Hall, H., Koschnitzhe, H. K., Stevens, B., and Wangensteen, O., *Surgery*, 1942, **11**, 333.