perimental pneumococcal infection. The results of bacteremia studies suggest that optimal doses of cortisone operate by enhancing host resistance to the infectious agent. On the other hand, doses larger than 5 mg per rat per day lower the resistance of rats to this infection. Hence, in cortisone therapy, as in the case with certain other hormones, *optimum dosage* is important and this may vary with different conditions.

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Received September 4, 1953. P.S.E.B.M., 1953, v84.

Properties of Bovine Anti-Hemophilic Factor. (20631)

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The production of a purified and concentrated preparation of human anti-hemophilic factor (AHF) has been hampered by the marked instability of this substance. Such rapid deterioration of plasma AHF occurs with storage either at room or refrigerator temperature, that more than half the activity may be lost within 24 hours. Moreover, AHF is largely destroyed in the process of plasma fractionation. Cohn's fraction I is the most potent plasma derivative presently available; yet various lots of this preparation contain only 8-35% of the anti-hemophilic activity represented in fresh normal plasma(1).

Although some success has been reported in the stabilization of human AHF(2), another approach would be the preparation of an intrinsically stable AHF derived from animal sources. Bovine plasma is known to possess anti-hemophilic activity, and potent fractions have been prepared from it(3). Ac globulin, which is unstable in human blood is stable in bovine blood(4). Accordingly, it was hoped that the AHF of bovine plasma might show a stability corresponding to that of the bovine Ac globulin. This has proved to be the case.

Methods and materials. Collection of blood. All blood specimens were collected in untreated glass containers. One part of 0.1 molar potassium oxalate to 9 parts of blood served as the anticoagulant. Beef, hog, and sheep blood were obtained from freshly slaughtered animals. The plasma was separated within several hours of collection, and was stored in deep freeze until ready for use. Unless otherwise indicated, the AHF content of human plasma was tested within one hour of blood collection. *Prothrombin* was determined by the method of Ware and Stragnell(5) with slight modifications in the case of serum specimens as previously described (6). *Thrombin evolution* was determined in recalcified plasma according to the technic of Pitney and Dacie(7). *Assay of AHF* was accomplished *in vitro* by testing the ability of the unknown specimen to induce prothrombin

consumption in the blood of severe, established hemophiliacs who had received no blood or plasma therapy for at least 2 months prior to testing. The details of this method have been published by Spaet and associates (6). In brief, 0.1 cc of the appropriately diluted specimens to be tested was added to 2 cc of hemophilic whole blood, and serum prothrombin content of the resulting mixture determined 4 hours after clotting. Serial dilutions of each blood or fraction were used, and AHF activity was estimated according to the concentration required to elicit 50% prothrombin consumption in the abnormal blood. In no case was any prothrombin consumption demonstrable when 0.1 cc of saline was added to the hemophilic blood, nor was the serum prothrombin ever lower than that of the respective plasma. *Cohn's fraction I* of bovine origin was kindly provided by the Cutter Laboratories, Berkeley, Calif. *"Acid globulin"* was precipitated from plasma by the technic of Ratnoff and Conley(8). *"AHF fraction"* was prepared in the following manner: Bovine fraction I was dissolved in isotonic saline so that the volume was equal to that of the original plasma from which it had been derived. This solution was brought to a temperature of 56°C and maintained at this temperature for 5 minutes to precipitate the fibrinogen. The precipitate was removed by centrifugation and the supernatant solution was absorbed with freshly prepared barium sulfate. Eight tenths moles of barium sulfate were used for each 100 cc of solution, and absorption was allowed to proceed for 30 minutes at a temperature of 37°C. The barium sulfate was removed by centrifugation, and Seitz filtration. The supernatant fluid was then diluted with 19 volumes of distilled water. This mixture was acidified to a pH of 5.4 with 1% acetic acid, as determined by testing in a Beckman pH meter, thus precipitating the active fraction. The precipitate was collected by centrifugation. This material is designated "AHF fraction," and will be thus referred to in the subsequent discussion. The precipitate was stored in deep freeze in its unmodified form, as solutions were found to be denatured by freezing. When ready for use AHF fraction was dissolved in isotonic saline buffered with one part in 10 of 1/15th molar phosphate buffer of pH 7.4. In general, solutions of AHF fraction were made up so that they represented the original volume of bovine plasma from which they had been derived. *Electrophoretic patterns* of plasmas and their derivatives were obtained on filter paper by a modification of the method described by Kunkel and Tiselius(9).

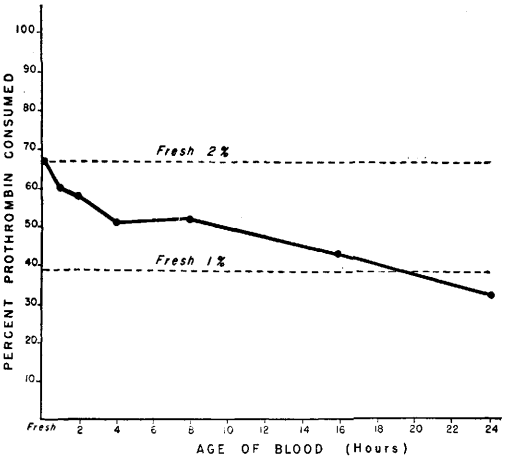

FIG. 1. Loss of AHF activity in human plasma at room temperature. A 2% concentration of plasma was added to hemophilic blood.

Results. Stability studies. Normal human blood was collected from a single donor at intervals as indicated in Fig. 1. The blood samples were each divided into 2 aliquots, one of which was stored at 20°C and the other at 4°C. These blood samples, representing various periods of storage, were simultaneously tested for their ability to restore prothrombin consumption to the blood of a known hemophiliac, which had been collected in a single syringe. Fig. 1 shows the deterioration of AHF activity in stored human blood. It can be seen that there was loss of more than half the activity in 24 hours, and that significant changes were to be found within 4 hours. In the case of this donor, the refrigerated specimens showed essentially an identical pattern of AHF loss, although other investigators have reported improved stability in the cold(10). In contrast, beef, hog, and sheep plasma showed no loss of AHF activity after 24 hours of storage at either 20°C or 4°C. Table I shows the AHF con-

TABLE I. AHF Activity of Human and Animal Plasmas in Hemophilic Blood.

Plasma	Conc. producing 50% prothrombin consumption (%)	Protein content (g/100 cc)
Fresh human	1	6.8
Bovine	.5	6.2
Sheep	.4	5.8
Hog	.2	6.0

TABLE II. AHF Activity of Plasma Fractions in Hemophilic Blood.

Fraction	Conc. producing 50% prothrombin consumption (%)	Protein content (g/100 cc)
Human fraction I (Cutter)	10	.4
" " " (Squibb)	7	.4
" " " (Mich. State Health Dept.)	8	.4
Human acid globulin	2	.8
Bovine " "	1	.9
" fraction I	1	.4
" AHF fraction	1	.1

tent in the various types of plasma studied. None of the human or animal sera had any appreciable AHF activity.

Fractionation. Table II shows the AHF activity of different plasma fractions. Three lots of human fraction I were assayed, each having been produced by a different manufacturer. None of these had more than 15% of the activity of fresh plasma, when considered on the basis of reconstitution to the original volume of plasma represented. On the other hand, bovine fraction I had about half the activity of the original plasma. A similar trend was seen in the acid globulin fractions of the various species tested. Preparations of beef, hog, and sheep plasmas had significantly more activity than that of human origin. The yield of AHF activity in the acid globulin derived from bovine fraction I (AHF fraction) was almost quantitative, despite a considerable reduction in the protein content of this preparation. A further check on the thromboplastic activity of this preparation was accomplished by means of the thrombin evolution test. Small amounts of beef AHF fraction were added to hemophilic plasma, and improvement of thrombin evolution following recalcification was compared to that produced by fresh human plasma. Reconstituted to the original volume of plasma from which it had been derived, the

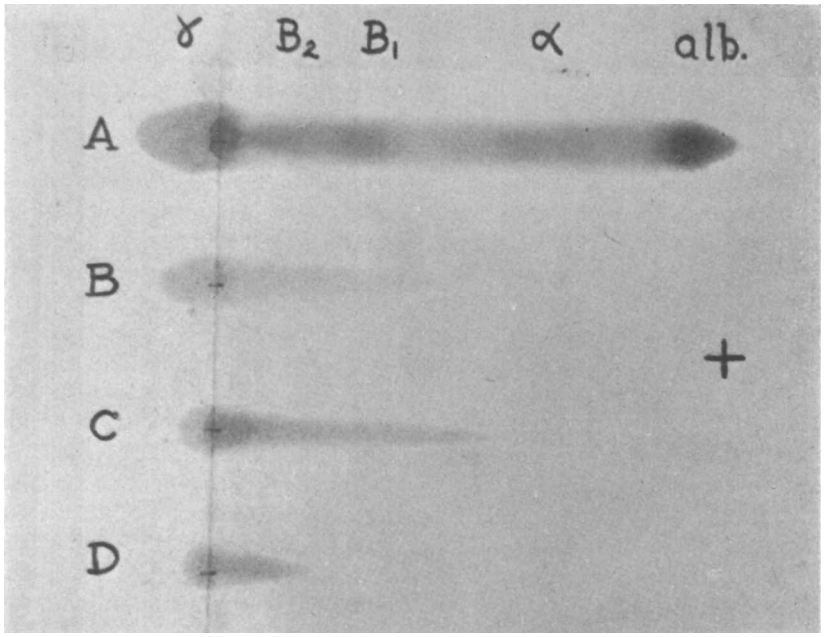

FIG. 2. Paper electrophoretic patterns of bovine plasma fractions. (A) whole plasma; (B) heated fraction I; (C) heated acid globulin; (D) AHF fraction (concentrated 5-fold).

beef AHF fraction was as active as whole human plasma. This finding indicates that the improved prothrombin consumption induced by beef AHF fraction is a true improvement of clotting, and not a nonspecific action resulting in loss of prothrombin. Fig. 2 shows the paper electrophoretic patterns of the various bovine fractions. Both fraction I and acid globulin were mixtures of α_2 and β globulins. AHF fraction was virtually pure β_2 globulin. It is evident that AHF fraction represents a considerable gain in both AHF activity per mg of protein, and purity as compared to previous preparations.

Beef AHF fraction contained no demonstrable fibrinogen, as tested by the addition of purified thrombin (Parke Davis). Neither was there any demonstrable prothrombin, or proconvertin (method of Owren and Aas)(11). Traces of labile factor activity were found, tested by the method of Quick(12). PTC activity(13) was kindly assayed by Dr. Paul M. Aggeler. Although beef AHF fraction was able to shorten the clotting time of *PTC*-deficient blood, 25 fold concentrations did not elicit any improvement in prothrombin consumption. In the precipitated form, beef AHF fraction was stable for at least 3 months in deep freeze; but freezing solutions of the material caused precipitation of the protein with complete loss of AHF activity.

Summary. The anti-hemophilic factor (AHF) of human plasma is unstable to storage, and shows great loss with attempts at fractionation. Bovine AHF is stable to storage, and protein fractions can be obtained which show a good yield of AHF. Beef "AHF fraction" was prepared as an almost pure β_2 globulin, and had about 70 times the AHF activity of fresh human plasma per mg of protein.

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Received September 14, 1953. P.S.E.B.M., 1953, v84.

Particle Size of Soluble Antigen of Rabies Virus. (20632)

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A study of the size and the antigenicity of the complement fixing "soluble antigens" of viruses may throw considerable light on virus structure. Recently Hoyle, Reed and Astbury (1) have shown that influenza virus can be broken down by ether treatment into complement-fixing and haemagglutinating particles. They obtained pure preparations from allantoic fluid and by electronmicroscopy estimated the particle diameter to be 12 mμ.

We have obtained the soluble antigen of rabies virus from infected suckling mouse brains. The particle size could not be determined in the Spinco preparative ultracentrifuge using the angle head (Polson and Linder (2)) because the amount of sedimentation