High-potency Antihæmophilic Factor Concentrate prepared from Cryoglobulin Precipitate

Investigations made to determine the causes of loss of antihæmophilic factor (AHF) during the Blombäck process[1] for preparing AHF-rich fibrinogen from large volumes of plasma have revealed that when the starting material is frozen plasma instead of fresh plasma there is a presumed liability which can be converted to an advantage.

The well-known susceptibility of AHF to denaturation by heat, storage, freezing and thawing has led to attempts both in Sweden[1], and in the United States[2], to arrange for prompt processing of freshly collected plasma whenever possible. If large-scale production is contemplated, however, such a requirement is a distinct handicap, and so our efforts have been directed to maximizing the yield from frozen plasma.

In order to investigate the causes of poor final product activity after standard Blombäck processing, samples were taken at each step to determine where the largest losses were occurring. As previously reported[3], the 'starting material' was found to have on the average only 54 per cent of the activity of the mean of normal individuals, and this low level was attributed to the freezing, thawing, and storage involved. Essentially all the remaining activity was recovered in Cohn Fraction I, but the citrate–ethanol–glycine extraction used to obtain the Blombäck Fraction I–O regularly resulted in large and variable losses of AHF activity. Still further decreases occurred during sterile filtration and ultra-violet irradiation. These latter procedures are not necessary in Sweden and England, where it is possible to process small batches under sterile conditions. Our final product, at that time, when prepared as a 2 per cent solution, typically had only the same AHF activity as normal plasma. More recent lots have approximately twice as much AHF activity, but even though the protein concentration is about one-third that of plasma, this is clearly a far from ideal therapeutic material.

A recent review of this project revealed that the 'starting sample' for analysis had in each case been taken after a preliminary step. This step, 'Clarification', involved centrifuging the plasma which had been thawed at 10°–12° C, pooled, and re-cooled to 1°–2° C, in order to remove any materials which had failed to dissolve. When six new batches of approximately 1,000 l. each were studied, it was found that the samples taken before clarification (the cryoglobulin material dissolves on warming for assay) averaged 90 per cent AHF activity, whereas after clarification the average was only 46 per cent. It thus became apparent that storage losses were not so great as previously thought, but that almost half the plasma AHF was being discarded in the clarification residue. This finding should not have been a great surprise since one of us reported previously that undissolved cryoglobulins in plasma include large amounts of AHF[4]. Attention now turned to this material, a gelatinous paste, of which approximately 2 kg were obtained from a 1,000-l. plasma pool. On a wet weight basis, between 10 and 20 per cent assays as protein, thus representing only about 0·4 per cent of the original plasma proteins. However, about 20 per cent of the original plasma AHF (half the AHF lost during the clarification) can be recovered from this material. Therefore, on a protein basis, a 50-times concentration of AHF activity has been achieved with this step alone.

Table 1. Circulating AHF Response to Fraction *PR* 2018 in Four Patients with Severe Classical Hæmophilia

Patient	^{51}Cr—Det'd. Plasma volume (ml.)	Fraction transfused (mg.)	Post-transfusion AHF-level (%)	Calculated AHF activity of fraction (units/g)
T. T.	2,500	375	16·5	870
R. P.	2,300	750	40	945
J. G.	2,360	750	35	848
S. F.	2,100	750	30	638
			Average =	825

Subsequent studies have dealt with further purification and sterile filtration of this material to prepare a concentrate for clinical use. A report on the details of this process is in preparation. The final product at present studied in clinical trials has an activity of 825 units of AHF (1 unit = AHF activity in 1 ml. of normal human plasma) per gram of protein, as determined *in vivo* after transfusion into hæmophilic patients. Actually our *in vitro* determination gives approximately twice this value in the Pool and Robinson AHG assay[5] following dilution of 0·1 ml. of the fraction with 0·9 ml. of hæmophilic plasma prior to the adsorption step. At this time there is no way of telling whether the true activity is in the range of 1,650 units/g, and, for some reason, only half of it is recovered after transfusion, or whether the true activity is 825 units/g, and we have not yet solved the problems of *in vitro* assay of very high potency materials.

Table 1 gives the data on the four patients with severe classical hæmophilia who received this material after determination of their chromium-51 plasma volumes had been carried out. The result on patient R. P., who received 750 mg of material, shows that a solution which can be administered with a 50-ml. syringe is capable of raising the circulating level of such a patient to 40 per cent of normal.

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[1] Blombäck, M., *Arkiv. Kemi*, **12**, 387 (1958).
[2] McMillan, C. W., Diamond, L. K., and Surgenor, D. M., *New England J. Med.*, **265**, 224 (1961).
[3] Ninth Cong. Intern. Soc. Blood Transfusion, Mexico City (1962).
[4] Pool, J. G., and Robinson, J., *Brit. J. Hæmat.*, **5**, 24 (1959).
[5] Pool, J. G., and Robinson, J., *Brit. J. Hæmat.*, **5**, 17 (1959).

IMMUNOLOGY

Specific Precocious Protective Action of Toxoids

There have been several reports of a specific precocious protective action, not involving antibody response, of tetanus toxoid against tetanus toxin[1–8]. Massive doses of tetanus toxoid injected into mice protected them against a few lethal doses of tetanus toxin injected at the same time, or shortly before, or shortly after. Diphtheria toxoid, given in doses comparable on a basis of antibody-combining power units, had no such protective effect against tetanus toxin[5,6]. Suggestions have been made that the protective action of tetanus toxoid may be connected with its ability to prevent the fixation of tetanus toxin by nervous tissue[7–9] and ganglioside[10].

We have tested whether other toxoids have a similar protective action against their homologous toxins. A number of toxins were titrated by injecting 0·5 ml. volumes of serial two-fold dilutions into control and toxoid-treated animals. Toxoid and toxin were injected in immediate succession intramuscularly in the same hind-limb. In the case of tetanus, toxoid and toxin were also injected intraperitoneally and intravenously. The toxins and toxoids (in crude form) were kindly supplied by Dr. R. O. Thomson of the Wellcome Research Laboratories. In each case, the largest practicable dose of toxoid was used, which was 0·5 ml. of the solutions as