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Observations on Plasma Banking and Transfusion Procedures for Haemophilic Patients using a Quantitative Assay for Antihaemophilic Globulin (AHG)*

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MANY clinicians have been puzzled by the failure of haemostasis in the haemophilic patient who has been transfused with apparently adequate amounts of freshly frozen plasma. Such failures become understandable, however, if a distinction is made between the amount of plasma administered and the amount of antihaemophilic globulin (AHG) administered. Information on the AHG levels in commonly used transfusion materials, on the AHG level required for haemostasis and on the survival of transfused AHG would permit rational planning of treatment. An essential requisite for such information is a reliable quantitative assay for AHG.

In the preceding paper (Pool and Robinson, 1959) a technique of assay is described which gives a coefficient of variation of less than 8 per cent ($\lambda = 0.0366$). Evidence is also presented from transfusion experiments that it is AHG and only AHG which is being measured. These same transfusion experiments cast doubt on the validity of the two commonly offered explanations of the inefficacy of transfusion. One of these postulates an abnormally rapid disappearance, within minutes, of transfused AHG, and the other proposes the presence of powerful anticoagulants in all severe haemophilic patients.

The present report describes studies which indicate that it is the low AHG levels in the transfused plasma and the short (10-hour) half-time of the AHG in the recipient's circulation that are responsible when therapy is ineffective. It also presents data on the causes of the low AHG levels in blood-bank frozen plasma and an analysis of the advisability of prophylactic transfusion therapy in haemophilia. It does not present any new information on the AHG levels required for haemostasis.

AHG LEVELS IN FRESHLY FROZEN PLASMA 'UNITS'‡ SUPPLIED BY THE BLOOD BANK

All the 'units'‡ used in this study were supplied in the routine manner for haemophilic patients who required transfusion. The units were usually 2–3 months old, since 3 months was the maximum storage time in this bank for individual units of frozen plasma and the oldest stock was used first. The sample for assay was taken immediately from each unit after thawing and thorough mixing by agitation. AHG levels in twenty-five such freshly thawed units of freshly frozen plasma were measured between December 1955 and August 1956, with the following results: 75 per cent§ AHG, 3 units; 50–75 per cent, 7 units; 25–50 per

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‡ A 'unit' of plasma is that amount obtained by processing the standard blood donation, 480 ml. of blood collected in 120 ml. of acid-citrate-dextrose (ACD). There are 275–300 ml. of citrated plasma in the frozen bank unit.

§ As compared with the level in our standard normal donor, previously shown to have a value close to the normal human mean. It must also be pointed out that during collection the blood is diluted with one-quarter of its volume of ACD. This routine blood-bank procedure thus results in an immediate dilution of the donor's AHG that is greater than that which occurs in the regular laboratory test. In the latter case nine parts of blood are diluted with only one part of 3.8 per cent trisodium citrate. Our standard donor whose level in the laboratory is 100 per cent would thus give a value of 85 per cent in a donated bank unit.

cent, 15 units. Since these low levels might be accounted for by loss in preparation of the plasma, in the storage period before delivery or in the thawing process before transfusion each of these factors was investigated.

Loss of AHG during Preparation of Plasma

Blood is collected by gravity into plain glass flasks containing 120 ml. of ACD solution, with occasional agitation of the flask. When four flasks have been filled, they are taken to another room and placed in a centrifuge which is not refrigerated. Although they are not pre-cooled, about 375 ml. of ice-water is poured into the centrifuge cup around each bottle. The centrifuge requires 15 minutes to reach 2000 r.p.m., 1 hour for the blood to be centrifuged at that speed, and another 10 minutes to come to rest. On one occasion the water around the bottles was found to be 19° C. at the end of this time. The bottles are then carried to a third room where the plasma is siphoned off into new flasks under aseptic conditions, after which the flasks are placed at −20° C. About 5 hours are required for the units to freeze solid. The time which elapses between each of these steps is necessarily quite variable since the technicians have other duties which often prevent prompt attention to the plasma.

For this study, four units were followed during collection and processing under the best possible conditions at the blood bank. Five days later they were all completely thawed in a water-bath at 37° C., sampled, and transfused to one recipient.

The AHG levels of the donor's plasma, at different times during collection, separation, storage, and administration, were ascertained in the following way. In each case, a donor set was provided with a Y-tube so that the first sample could be taken as the blood was flowing into the flask; this sample was mixed with one part of 3.8 per cent (w/v) trisodium citrate to nine parts of the blood and centrifuged for 10 minutes at 3000 r.p.m. The plasma was immediately pipetted off and placed at −20° C. for later assay. A second blood sample was taken from each filled flask with a sterile syringe just before the bottle was placed in the centrifuge; the plasma from the sample was immediately separated by centrifugation for 10 minutes at 3000 r.p.m. and then frozen. The third sample of plasma was obtained in each case from the siphon used to fill the bank's plasma flask at the end of the centrifuging, and it, too, was promptly frozen. The fourth sample came from the plasma flasks when they were all thawed before transfusion; these samples were also frozen. All sixteen samples were thawed and assayed the next day (Table I).

TABLE I

AHG LEVELS DURING COLLECTION AND PROCESSING

	1st unit (%)	*2nd unit* (%)	*3rd unit* (%)	*4th unit* (%)
AHG level in donor	142	127	175	41
AHG level in bottle before centrifugation*	115	108	170	40
AHG level in plasma after centrifugation, before freezing	71	66	120	40
AHG level in plasma after thawing	67	52	117	26

* A fall of 15 per cent is caused by dilution with the anticoagulant.

If the error in the method (8 per cent) and the dilution factor (15 per cent) are both considered, a 23 per cent fall in AHG level between the first and second samples for each unit

could be accounted for. Since the largest decrease observed (1st unit) was from 142 to 115 per cent (a 19 per cent fall), we conclude that probably no AHG is lost during the collection. However, the plasma is not further diluted after this point and the 8 per cent error in the method cannot thus explain the losses of 38 per cent, 39 per cent and 29 per cent in the first three units during centrifugation. The AHG concentration in the fourth unit, already low, did not fall further. In two of the four units the concentration fell still further during the freezing and thawing processes. Apparently therefore the most hazardous period for the plasma AHG is during centrifugation. The final relative concentration of AHG in the four units paralleled that originally present in the donors' plasma. Selection of donors with high initial levels would thus tend to assure a relatively high level of AHG in the frozen plasma.

Loss of Plasma AHG at Different Temperatures

The most probable cause for the fall in AHG levels during centrifugation is the rise in temperature. To investigate this, plasma samples from one donation were stored for a period of 6 hours at five different temperatures. The AHG in each sample was as follows:

Frozen (−20° C.), 100 per cent; refrigerated (4° C.), 82 per cent; room temperature (20° C.), 66 per cent; 37° C., 14 per cent; 56° C., 3 per cent.

The data confirm the results of Penick and Brinkhous (1956) and others. The findings also account for much of the loss of AHG when the processing of the plasma takes place at temperatures exceeding 4° C.

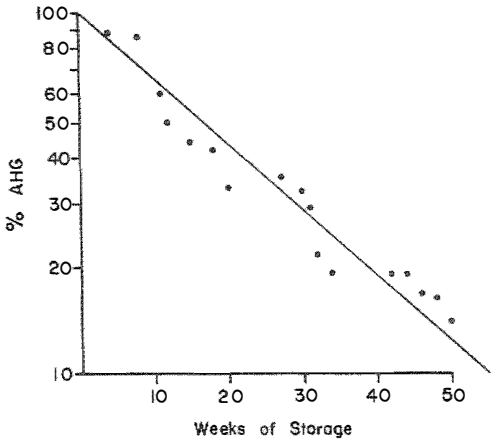

FIG. 1. Deterioration of AHG in frozen plasma.

Deterioration of AHG in Frozen Plasma

Successive samples of citrated plasma obtained from the same donor over a period of 1 year were stored at −20° C. and then all assayed for AHG on the same day. Although these samples were collected using a different volume and kind of anticoagulant from that used by the blood bank, comparisons between the samples from the same donor using the two different proportions and types of anticoagulant indicate that the difference in AHG level can be accounted for entirely by the different dilution factor. Fig. 1 shows the AHG deterioration curve: the half-life is about 16 weeks, suggesting that if the blood bank stores plasma units for 4 months, half of their AHG content will by then have disappeared.

minutes, using a one-tenth volume of 4 per cent sodium citrate as anticoagulant; the blood is immediately chilled with wet ice, and transported to the laboratory within half an hour. Centrifuging in a refrigerated centrifuge, drawing off and pooling of plasma, clarification through a continuous centrifugal clarifier, irradiation, filling into the final containers, and shell freezing* are all carried out in rapid succession so that no more than 5–6 hours are consumed for the entire process from the time the first blood is drawn from the group of 20–24 donors. The frozen plasma is placed in the driers within 24 hours of the time it is frozen. The values for four of the units assayed at the same time were examined statistically to determine whether the observed amount of variation would be expected, taking into account that normal plasma varies between 50 per cent and 200 per cent in its AHG content and the fact that blood from twenty donors was pooled for each lot. The spread is too large to be accounted for by the variation among donors, which suggests that even the expeditious Hyland processing allows significant variation in the preservation of AHG.

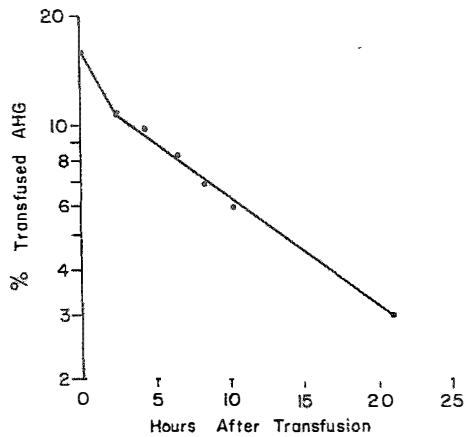

FIG. 2. Disappearance of transfused AHG.

SURVIVAL OF AHG TRANSFUSED INTO THE BLEEDING HAEMOPHILIC PATIENT

The disappearance of transfused AHG from the plasma of a patient (J. G.) was followed after the administration of 4 units of plasma. The data form a line with an early, steep slope, followed by a later more gradual slope (Fig. 2). The gradual slope fits a semi-logarithmic plot and gave a half-time for the transfused AHG of 10.2 hours. A similar study in another patient (W. D.) was complicated by the plasma not being given all at once. Only three observations could be made afterwards, but they nevertheless fitted a similar disappearance curve. The half-time of 10.2 hours may be compared with the 4 hours estimated by Brinkhous and co-workers (1956) and 11 hours by Biggs (1957).

Two principal processes probably account for the disappearance of transfused AHG: transport across capillary walls and degradation of the material. Since AHG is a globulin, the figure of 3–4 per cent per hour, reported for the rate of vascular–extravascular transfer

* The term 'shell freezing' is used by Hyland Laboratories and others to describe a process whereby a liquid is rapidly frozen on the inner surface of a turning vessel in a relatively thin layer, rather than being more slowly frozen into a solid mass in a stationary container.

of transfused ^{131}I-labelled γ-globulin, may be relevant for the first process (Slater and Sass-Kortsak, 1956). This could account for a 30–35 per cent loss in 10 hours, leaving 15–20 per cent to be explained by degradation. However, another more rapid process appears to be superimposed on these two in the period immediately following the transfusion. At the present time one can only speculate that this may result from loss of AHG in clotting at the bleeding site or from adsorption of AHG to platelets and vessel walls. It should perhaps be pointed out that we have no idea why the half-life of AHG at 37° C. in the patient is about 10 hours whereas its half-life at 37° C. in the test tube is only about 2 hours.

THE PROBLEM OF PROPHYLAXIS

Information about the average AHG level in freshly frozen plasma units, about the survival of administered AHG, and about the AHG level required to prevent bleeding permit a mathematical analysis of the advisability of prophylactic transfusion. If a patient is transfused daily with 300 ml. of plasma the patient's AHG level will gradually rise to a plateau level. Assuming the half-life of transfused AHG to be 10 hours and the percentage of AHG in the average bank unit of frozen plasma to be 50 per cent, just before his transfusion each day the patient's AHG level would be 1.15 per cent and just after his transfusion the level would become 6.15 per cent. These are the quantities provided by the transfusion; 1–2 per cent AHG might be added to each figure to allow for endogenous AHG if we could estimate it reliably.

A prediction of the efficacy of prophylactic transfusion must depend on a comparison of the results of the above kind of calculation with the level believed to be necessary for haemostasis. Estimates of the latter are the weakest factor in the rational planning of treatment. They range from 5 per cent (Langdell, Wagner and Brinkhous, 1955) to 30 per cent (Macfarlane *et al.*, 1957) but in all cases these are the suggested minimum concentrations below which the level should not drop. In our example calculated above, our patient would be above the minimum suggested level, 5 per cent, for only 3 hours out of the 24. Thus, at the most, a daily unit of blood-bank plasma would protect the patient one-eighth of the time and would consume large amounts of material. If the average (Hyland) lyophilized unit, which has 72 per cent AHG, was used instead, the patient's AHG concentration would still reach only 8.9 per cent after the transfusion each day and remain above 5 per cent for only 8½ hours of the 24.

Thus, with the materials now available, even a daily transfusion gives a haemophilic patient no assurance of 24 hours' protection. When the expense of such a programme and the risks of sensitization to AHG and of homologous serum jaundice are all considered, it is impossible to recommend a prophylactic regime. In the treatment of bleeding episodes the most urgently needed information is the circulating level of AHG required for haemostasis. This level probably varies greatly with the site and degree of bleeding and studies are needed to correlate observations on successful haemostasis with the circulating AHG concentration in a variety of patients and in a variety of situations. Then the calculation of the initial transfusion quantity and the maintenance doses required might be made with considerable confidence.

SUMMARY

Most 'units' of freshly frozen plasma supplied by the local blood bank after no more than 3 months' storage have less than 50 per cent of the mean AHG level of the donors.

Up to half of the donor's AHG is lost during the centrifuging process for separation of the plasma; the half-life of the remainder in the wet-frozen state is about 16 weeks. If any cold-precipitated fibrinogen remains behind after thawing and administration of a plasma unit, it indicates that significant amounts of the unit's AHG have also been precipitated and were thus not administered.

Seven different lots of (Hyland) commercial, frozen, lyophilized plasma contained on an average 72 per cent of AHG (range, 46–103 per cent).

After an initial rapid fall the AHG transfused to a bleeding haemophilic patient disappeared exponentially; its half-life was 10.2 hours.

Mathematical analysis shows that the daily transfusion of an average blood-bank plasma unit to a haemophiliac would keep the circulating AHG level above 5 per cent for only 3 hours out of the 24. Daily infusion of an average 250 ml. frozen-lyophilized unit (Hyland) would extend this time to only 8½ hours.

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