Brief Report

Factor VIII (AHF) Concentration in pH 6.5 Citrate-Plasma

By Francis S. Morrison

As a result of many recent investigations into the effect of anticoagulants on the survival of transfused platelets,\textsuperscript{1,2} the fact has emerged that ACD is the anticoagulant of choice for platelet transfusion. The quantities of platelets which are usually required clinically can be most conveniently provided as platelet concentrates.\textsuperscript{12} The suggested technics for making platelet concentrates require reduction of pH of the platelet rich plasma (PRP) with additional acid citrate; consequently, a by-product is fresh ACD-plasma at pH 6.5. Many laboratories might find it convenient to store this plasma for use in the treatment of classical hemophilia. Therefore, the effect of the additional ACD on Factor VIII (AHF) concentration was investigated.

Methods and Materials

The Factor VIII assay used was the one-stage method of Langdell, Wagner and Brinkhous\textsuperscript{13} as modified by Hardisty and Macpherson.\textsuperscript{14}

Ten volumes of whole blood were collected from normal volunteers into 1.5 volumes of ACD\textsuperscript{*}. Platelet-poor plasma (PPP) was obtained by centrifuging at 1800–1900 g for 30 minutes. After the separation of PPP, three different procedures were used:

1. The PPP was partitioned into two plastic tubes. One tube contained 0.3 ml. additional ACD; the other contained the same volume of Veronal buffer (pH 7.3). Aliquots of these samples were assayed for Factor VIII activity before and after storage at −20°C. The pH of the samples with added ACD ranged from 6.3 to 6.5 while the buffered plasma ranged from 7.0 to 7.2.

2. It was felt that the reduced pH and/or additional citrate might affect the assay system quite separately from any effect on Factor VIII. In an attempt to circumvent this possibility the buffering capacity of erythrocytes was utilized. The PPP was treated as described in the preceding paragraph, but immediately prior to assay it was returned to an equal volume of fresh-washed erythrocytes. The control plasma (pH 7.0–7.2) was treated in the same manner as the "acidified" plasma (pH 6.3–6.5). After standing at room temperature for 15 minutes the plasma was again separated and then assayed. The pH was determined before and after the incubation with erythrocytes.

3. Another method used was based on the Factor VIII cryoprecipitation technic of Pool and Shannon.\textsuperscript{15,16} The PPP was partitioned into three portions of approximately 2 ml. in

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*ACD NIH Formula A (0.8 per cent citric acid, 2.2 per cent sodium citrate, and 2.45 per cent hydrous dextrose).
small plastic containers. To the first was added 0.2 ml. more ACD, while Veronal buffer, 0.2 ml., was added to the second and third samples. The third sample was then stored at −20 C. The first and second samples were rapidly frozen by immersion into an alcohol “dry ice” mixture and were then allowed to thaw overnight at 4 C. and were centrifuged at 2000 g for 20 minutes. After pouring off the supernatant plasma the cold-precipitate was stored at −20 C. for 1 to 10 days. When the assay was to be done, all three samples were taken out of the freezer, thawed at room temperature, and the cold-precipitate in the first two samples was resuspended in 2.2 ml. buffer.

RESULTS

1. When the pH of the samples differed at the time of assay, the estimate of the sample with the additional ACD was approximately 50 per cent (average 54 per cent) of the Factor VIII concentration of the sample without additional ACD.

2. When the plasma was returned to fresh erythrocytes and then separated, the pH of the sample with additional ACD did not differ from the sample without additional ACD by more than 0.2 units. When these samples were assayed, the Factor VIII concentration of the samples with additional ACD was found to be 90 to 100 per cent of the control (average 96 per cent).

3. With the cryoprecipitate technic, the final preassay pH of all three samples differed by less than 0.1 unit. The Factor VIII concentration of the plasma fractions which were thawed at 4 C. overnight was 40 to 60 per cent (average 52 per cent) of that in the sample stored at −20 C. immediately after separation. There was no difference, however, between the paired samples which were cold-precipitated, one of each pair having been processed in the presence of additional ACD and a pH approximating 6.5.

DISCUSSION

A reduction in plasma Factor VIII concentration of 25 to 30 per cent in acidified plasma has been recently ascribed to altered pH or salt concentration. However, it was not stated whether an attempt was made to readjust the pH and/or salt concentration prior to assay, nor was the method of assay given. In the studies reported here a comparable effect on AHF assay of the “acidified” plasma was seen. However, this difference was not seen when the pH was readjusted to physiologic levels prior to assay.

It is well known that pH can influence the thrombin-fibrinogen reaction. It appeared, therefore, that the lower Factor VIII assay was a reflection of the lower pH. However, the pH of the actual incubation mixture as well as the reaction mixture of the assay system was determined and found to be unaffected by the pH of the plasma being assayed. (Plasma is greatly diluted in buffer during the assay.) This does not rule out the possibility that reduced pH alters the Factor VIII molecule so as to result in a reversible reduction in activity. However, the reduction is not reversed at physiologic pH during assay. Alternatively, salt concentration is also known to influence the thrombin-fibrinogen reaction, and the improved results seen under the given experimental conditions may reflect the dilution or removal of citrate ion independent of the pH change. The data do not allow a definitive conclusion on this question.
The experiment utilizing the buffering capacity of erythrocytes was a simple attempt to simulate the in vivo events attendant upon infusion of plasma into a hemophiliac. Under these experimental conditions there was no significant reduction in Factor VIII concentration after exposure to, or storage in, additional ACD.

The recent development of the technic of cryoprecipitation of Factor VIII and its application to general blood banking may represent a large step forward in Factor VIII procurement. The end product is reported to be 30 times as potent as frozen plasma, on a quantitative protein basis. In the present study the cryoprecipitation method was used only as a means of eliminating the excess citrate and the reduced pH as factors in the assay system. The plasma precipitated in the presence of additional ACD had the same Factor VIII activity as the plasma samples precipitated without additional ACD. The results also indicate that the additional ACD does not interfere with the cryoprecipitation method of Factor VIII concentration.

**SUMMARY**

A by-product of the preparation of platelet concentrates, by recently proposed methods, is fresh citrate-plasma at pH 6.5. Because this plasma might be a useful source of Factor VIII, the activity was investigated. Compared to unaltered citrate-plasma, the activity was found to be 54 per cent. However, after incubation with washed fresh erythrocytes the "acidified" plasma had the same Factor VIII activity as the control.

This suggests that, after infusion, pH 6.5 plasma is probably as effective a source of Factor VIII as ordinary ACD-plasma. This conclusion was supported by observations on Factor VIII concentrates prepared by cryoprecipitation. In such preparations additional ACD and pH are eliminated as factors in the assay system. Factor VIII concentration in the precipitates prepared from acidified plasma was found to be as high as in precipitates prepared from untreated plasma.

**Summario in Interlingua**

Un producto secundari del preparation de concentratos de plachettas secundo recentemente proponite methodos es citratate plasma fresc a un pH de 6,5. Viste que iste plasma poterea esser un utile fonte de Factor VIII, iste activitate esseva investigate in illo. In comparation con nonalterate plasma a citrato, il esseva trovate que le activitate amontava a 54 pro cento. Tamen, post incubation con eluite erythrocytos fresc, le “acidificate” plasma hadeva le mesme activitate de factor VIII como le preparato de controlo.

Isto suggestiona que un plasma de un pH de 6,5 es probablemente post su infusion non minus efficace como fonte de Factor VIII que citratate plasma ordinari. Iste conclusion esseva supportate per observationes con concentratos de Factor VIII preparate per cryoprecipitation. In tal preparatos, le addition de citrato e le pH es eliminate como factores in le sistema de essayage. Esseva trovate que le concentration de Factor VIII in le precipitatos preparate ab plasma acidificate esseva non minus alte que in precipitatos preparate ab plasma nontractate.
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