REATIONS TO AN EDINBURGH FACTOR VIII CONCENTRATE

AN APPRECIATION

1. Early in September 1975 reactions occurred in two patients receiving FFC Factor VIII concentrate at the Royal Infirmary, Glasgow, the incidents occurring within a period of 24 hours. Clinical summaries are attached as an Appendix.

2. It is noted that:
   a. Both reactions were similar; after a latent period of about ½ hour after infusion the patients felt generally unwell and shivery. On examination they were sweating, suffering from uncontrollable muscular twitching and had tachycardia.
   b. Temperature was normal when checked in Patient 2.
   c. Patient I had a slight erythematous rash.
   d. In neither case was there any degree of hypotension.
   e. Recovery was rapid and complete after treatment with hydrocortisone and pririton.
   f. The infusions were from different batches; Patient I received two bottles of batch 89, Patient 2, four of batch 90. In each case pririton was given prior to the infusion, which was given over a period of 20-30 minutes.
   g. Patient I had required treatment, probably including commercial Factor VIII, cryoprecipitate and the FFC product 3-4 times weekly for the past two years at least and had previously complained of tiredness, headache and rashes after the latter preparation.
   h. Patient 2, also receiving various preparations, required a course of treatment lasting several days about once a month.

3. Following reporting of the incidents, the following steps were taken.
   a. Remaining units from the two batches (89 and 90) were withdrawn.

These two batches were made in the RIE, Edinburgh shortly before transfer of the FFC to Liberton, quality control and finishing arrangements being carried out at the latter site. Apart from bottles used in a trial administration in Jan-March 1975 (for details of this trial see para 7), the batches issued each consisted of 22 bottles and were sent to the R.T.C. for use by the Edinburgh and Glasgow Haemophilia Centres. 21 bottles of batch 89 and 13 of batch 90 were used clinically without adverse effects apart from the incidents under discussion.

b. A sample of the distilled water used to reconstitute the preparations in Glasgow was sent to the RTC Law for pyrogen testing; the result was satisfactory.

c. The Law Centre carried out a series of tests on the remaining material and on post-transfusion blood samples from the two patients. Salient results are:
   (1) Pyrogen testing on batch 90

Original quality control test before issue 4.2°C
Repeat 4.5°C, further repeat 3.5°C.
(2) As the patients were group AB and B respectively Anti-A and Anti-B allo-antibody titres were determined in the concentrates using known ABO group cells. Results at 37°C were:

<table>
<thead>
<tr>
<th>In saline medium</th>
<th>Batch 39</th>
<th>30</th>
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<tbody>
<tr>
<td>A 1 cells</td>
<td>1/64</td>
<td>1/128</td>
</tr>
<tr>
<td>B cells</td>
<td>1/32</td>
<td>1/64</td>
</tr>
<tr>
<td>Patients' cells</td>
<td>1/32</td>
<td>1/8</td>
</tr>
</tbody>
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**Indirect antiglobulin test**

<table>
<thead>
<tr>
<th></th>
<th>Batch 39</th>
<th>30</th>
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</thead>
<tbody>
<tr>
<td>A 1 cells</td>
<td>1/512</td>
<td>1/512</td>
</tr>
<tr>
<td>B cells</td>
<td>1/2048</td>
<td>1/256</td>
</tr>
<tr>
<td>Patients' cells</td>
<td>1/2048</td>
<td>1/32</td>
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(3) Indirect antiglobulin tests on the patients' cells were negative.

(4) In neither of the post-transfusion specimens from the patients was there any evidence of antibodies to leucocytes, platelets or red cell antigens other than the Anti-B in the Group A patient.

(5) The serum from patient 2 contained an antibody within the Gm system inhibitable by batch 30. This is a result which will be further investigated when fresh serum samples are obtained.

d. SHHD decided as a precautionary measure that the use of PPC F VIII for home treatment be discontinued for the meantime and requested that particular attention be paid to the occurrence of any reaction, however minor, in patients receiving treatment in hospital with this preparation.

It is also understood that the batch of distilled water used for reconstitution was withdrawn.

4. It is extremely difficult to pinpoint the exact nature and pathogenesis of reactions of this nature as the symptoms and signs (para 2a) are frequently non-specific. There are numerous causes of reactions to blood and blood products and one can discuss these in relation to these two incidents in terms of possibilities rather than the likelihood of arriving at definite conclusions. Little information is documented on reactions to blood products as opposed to those following transfusion of whole blood. I have been informed by a Haemophilia Centre Director that a moderate number of reactions occurred when fresh plasma was the mainstay of treatment; reactions, variable in extent and frequency, occurred in up to 50% of patients. When Cohn's fraction was in use some patients objected to it because of reactions experienced. Some 1/40 of those receiving cryoprecipitate have episodes of malaise, headache and sometimes rashes, but these are not alarming. Until the episodes in Glasgow during the period 1 Jan 1974 - 30 June 1975, 550 vials of PPC Factor VIII had been given without any reactions being reported. One has heard, without any factual detail, that reactions have followed the use of commercial preparations of Factor VIII.

5. The following types of reaction have been considered in this context:
   a. Febrile
   b. Pyrogenic

2.
c Infective

6. Febrile Mild febrile reactions, often unnoticed, commonly follow whole blood transfusion; in a few cases (some 2%) these are more marked, pyrexia being accompanied by chills. It is now generally accepted that in most cases this type of reaction follows sensitisation to leucocyte, platelet or plasma proteins by previous transfusion and are in actual fact examples of allergic reactions (para 10).

7. Pyrogenic Pyrogens are bacterial polysaccharides of high molecular weight and are derived from dead organisms in contaminated fluids or apparatus. Quality control includes estimation of pyrogenic activity by means of a rabbit test, the aggregated rise in temperature of 3 rabbits injected intraperitoneally with the material being recorded. BP standards, a maximum aggregate rise of 1.15°C, were originally laid down some 25 years ago for penicillin solutions and are widely used for quality control of crystalloid fluids. The test, and standards, have been empirically adapted to protein fluids such as Factor VIII, but I do not know of any work which correlates the temperature reaction in rabbits to pyrogen content of a proteinaceous fluid. Some preparations of Factor VIII do produce in rabbits temperatures higher than the BP standards and attempts have been made to assess the significance of this in clinical terms.

It is known that preparations of Christmas (IX) factor, giving an aggregate temperature rise in rabbits of 3°C give results in the region of 0.5°C when retested after heat treatment, but as heating does not destroy pyrogens some other factor must be affecting the test animals. Similar tests cannot be performed on Factor VIII as it gels on heating and hence is unsuitable for injection.

To avoid unnecessary rejection of valuable material, ten bottles from different batches of Factor VIII with rabbit pyrogen test results ranging from 2.4°C to 4.4°C were issued to both the Edinburgh and Glasgow centres early in 1975 with the knowledge of, and cooperation by, the haemophilia centre directors. Clinical results of this test series of 20 bottles were negative, in that all the units were used without any adverse reactions. This test series included samples from batch 89 (rabbit pyrogen test 3.8°C) and 90 (4.2°C).

It was estimated that issue of material up to 4.2°C would involve no more than a 5% loss for this particular quality control standard and Factor VIII issues have been made up to this figure of 4.2°C. Further refinement in technique by incorporating an additional wash at the final precipitation stage in fractionation has been introduced recently and appears to decrease reactivity in rabbits by 30-40%. Batches subsequent to 90 have mostly "passed" the rabbit pyrogen test. What deleterious factor (in terms of rabbit response) is removed by this additional wash is unknown.

An alternative pyrogen test using material from crabs (the limulus test) has been recently introduced and was negative in both batches 89 and 90. Experience with this test, in particular the possible occurrence of false negative results, is still limited however and it is used as an ancillary to, not a substitute for, the rabbit test.

A pyrogen reaction is characterised by a latent period before the onset of symptoms, initial clinical features are non-specific and include chilliness, nausea, headache and backache followed by the essential feature (as the name implies) of pyrexia with flushing and sweating. There is either no change, or a rise, in blood pressure. The plateau type of temperature response generally subsides within 8 hours. The response can be prevented or ameliorated by amidopyrin, but not by histamines.

The Glasgow incidents showed latency and non-specific symptoms, but in the
case where the temperature was recorded the patient was pyrexial and both
recovered rapidly.

In summary, although these preparations "failed" the rabbit test, it is
difficult to attribute these reactions to the presence of pyrogens both on clinical
grounds and on the fact that considerable quantities of the same and similar
preparations (over 50 vials) had been infused into other patients without
untoward effect. Dr. Bengham (Head of the Hormone and Blood Products Division of
the National Institute of Biological Standards) has expressed his view to Mr Watt
that human tests take precedence over, and nullify, the results of rabbit tests.

8. Infection Contamination of an intravenous fluid by living organisms, even
saprophytic, is fraught with danger, but, as concentrated Factor VIII is a freeze-
dried preparation tested for sterility and is given to the patient very shortly
after reconstitution, infection can be excluded on these grounds alone. Apart
from this the clinical features when infected fluid is transfused are those of
sudden collapse with profound hypotension.

9. Haemolytic While, in general, plasma or preparations derived from it, is so
diluted in the recipient's circulation that donor ABO antibodies are of comparatively
little importance, certain donors have a high titre of these antibodies which can
react with recipient cells and produce a haemolytic reaction. In view of this
factor, group O blood is screened for plasma agglutinin content and not transfused to
other groups unless the titre is below a certain level (1/160 is generally accepted
as being the dividing line); at Lew plasma for drying is made up in batches with
representative A, B, and O groups to provide a balanced agglutinin content; in some
centres cryoprecipitate is issued as known ABO groups. As regards concentrates of
Factor VIII, quality control includes estimation of A and B agglutinin content,
using saline techniques, the upper limit accepted being a titre of 1/64, very much
below any danger level in view of the total volume infused.

Repeat ABO antibody tests at Lew (para 3 c(2)), accepting a technical variation
of plus or minus one dilution, are satisfactory in a saline medium. The high titres
found using an indirect antoglobulin test are difficult to interpret. One would
expect a high IAG test to be mirrored by a high saline test, but this is not so in
this series. Some non-specific factor may be affecting the IAG test and it may
be noted that tests on a specimen of commercial VIII showed non-correlation between
these tests, titres being saline 1/8 and IAG 1/256. Although I do not consider that
the IAG test results are germane to this discussion, the matter is of interest and
similar tests will be made on other batches.

The usual haemolytic transfusion reaction when donor cells react with recipient
antibodies has a dramatic picture including flushing, pain in the lumbar region and
constricting pain in the chest, followed by evidence of haemolysis. In reactions
due to interaction between recipient cells and donor antibodies such acute mani-
festations of haemolytic shock are absent and the usual clinical picture is a
failure of the transfusion to achieve its aim. Progressive anaemia has been
reported in a haemophilia patient repeatedly infused with Factor VIII material
containing a high agglutinin titre to his red cell antigens.

The clinical picture in the Glasgow incidents is not that of haemolytic reactions.

10. Allergic (anaphylactoid) Any foreign protein and some substances conjugated
with protein (haptens) given parenterally can stimulate antibody production in the
recipient. Frequent transfusion of any sort (blood or blood products) will
inevitably subject the recipient to the risk of sensitisation. Two important
factors influence the extent and degree of this, the antigenic power of the
protein and individual reactivity. Fortunately it would appear that the non-
cellular elements of the blood are only weakly antigenic and only a minority of
patients frequently transfused with plasma proteins become sensitised, for
example only some 6% of haemophilia patients develop Factor VIII antibodies. In
Sensitized patients further exposure to the antigen may lead to an antigen-antibody reaction which in general causes a mild allergic (anaphylactoid) type of reaction characterized by skin manifestations (wheals), which if few in number can be overlooked, headaches, sometimes rigors and sometime pyrexia. In one reported series the incidence of mild anaphylactoid reactions following whole blood transfusion was 3%, but there is little factual information of the incidence following infusion of blood products. Again, fortunately, the weak antigenicity of proteins from the same species (man) determines the rarity of severe anaphylactic reactions, but I have been informed of such a reaction with a fatal outcome after infusion of whole plasma.

The non-specific nature of the symptoms and signs in the Glasgow reactions would fit in with the clinical picture of allergic reactions and patient allergy is more in keeping with the previous clinical experience of the two batches in question. Not too much stress should be placed on the finding that the serum of patient 2 contained an antibody within the Gm system, but it may be a pointer. The commonest cause of anaphylactic and probably anaphylactoid reactions is interaction between the globulin IgA and anti-IgA; no other antigen - antibody reactions involving normal plasma proteins have been clearly incriminated although a little evidence exists that anti-Gm in a recipients plasma may very occasionally cause reactions.

Two factors are known to prevent or reduce the severity of reactions in sensitised patients previous administration of histamines and giving the infusion slowly. In a haemophilia patient with a bleeding episode initial Factor VIII administration must be given sufficiently rapidly to achieve haemostasis, thus preventing further loss of coagulation factors, but once this has been achieved further infusion can be given more slowly.

Whether intradermal testing of a severe haemophilic requiring frequent treatment, each time a new batch of Factor VIII concentrate is received, in analogy with intradermal sensitivity tests prior to giving equine anti-tetanus serum, would be feasible and productive is a question which merits consideration.

11. Conclusions

a. The reactions reported in Glasgow were most likely of an allergic nature due to patient sensitisation following multiple treatment with plasma products.

b. Any preparation of Factor VIII, be it plasma, cryoprecipitate, commercial or NHS concentrate, contains unnecessary globulins, other proteins and platelet debris; continued research and development is directed towards eliminating these, but at present it must be accepted that occasional allergic reactions will occur in a minority of patients repeatedly receiving blood or blood products.

c. Clinical experience confirms the view that preparations producing aggregate temperature rises in the rabbit test up to 4.2°C do not lead to pyrogenic reactions in man.

d. Nevertheless, at present clinicians should be notified of the results of pyrogen tests for each batch issued so that they may, on the basis of their previous experience of this material, decide if they wish to limit the situations in which it is used.

e. The May and Baker vial for injection of the batch used for reconstitution in Glasgow was not pyrogenic.

f. A system of reporting reactions to any blood product, including negative findings, should be instituted in order to obtain documented evidence of the extent of the problem.