PLASMA FRACTIONATION LABORATORY, OXFORD

Report to Blood Products and Blood Group Reference Laboratory Managing Committee 1975.

Research and Development

Toxicity Tests: Work has continued on the assessment of suggested in vitro tests for thrombogenicity. The observation of Dr. Aronson that inclusion of a small amount of heparin in the material prevented the development of clots in the in vivo test ("Wessler") described in the last report was confirmed. At its meeting in Basel, September 1974, the Task Force on the Clinical Use of Factor IX Concentrates of the International Society for Thrombosis and Haemostasis recommended that heparin (1 i.u. per 10 u of factor IX) should be added to factor IX concentrates before sterilisation. Although there has been no clear-cut evidence of thrombogenicity associated with the use (including use in 38 major surgical operations) of more than 12.25 million units of factor IX prepared in Oxford by the DE(1) method up to the end of 1974, it is realised that the concentrate may be used occasionally in patients suffering from conditions other than deficiency of a single clotting factor. It was, therefore, thought prudent to accept the advice of the Task Force and the recommended amount of heparin is now added.

At this same meeting of the Task Force three new in vivo tests were proposed and discussed:-

(i) The effect of factor IX concentrate on the recalcification time of platelet-poor plasma (Kingdon,H.S.);

(ii) A test based on the generation of thrombin in a recalcified sample of factor IX concentrate (called the TGT_{50} test of Sas, G.);

(iii) The effect of factor IX concentrate on the recalcification time of plasma exhausted by six treatments with Celite (Sas, G.).

No conclusion was reached concerning the nature of the thrombogenic material; it was suggested that it might be activated factor IX (factor IXa), activated factor XI (factor XIa), or activated factor X (factor Xa); another suggestion was that the thrombogenic effect might be due to traces of thrombin (factor IIa).

In our hands the test described by Kingdon was not reproducible. The substrate was unstable even when stored in liquid nitrogen in the presence of a stabilising buffer (HEPES); the reagents were unstable at room temperature during the test and had to be kept in an ice bath. Many workers made similar observations at the Task Force meeting in Paris in July 1975.

We discussed the other proposed tests with [redacted] and who pointed out that normal plasma for transfusion would fail the TGT_{50} test of Sas. It was felt that this test would be unduly sensitive to traces of clotting factors such as factor V, or even factor VIII, which the recipient of the material would have in his circulation. Extensive investigation in our laboratory showed that material which we knew was clinically safe would not pass the TGT_{50} test so that it seemed to us that it was not relevant as a safety test for factor IX concentrate.

The instructions for the preparation of Celite exhausted plasma required six treatments with the adsorbent which would remove not only factor XI, as intended, but other factors, especially factors V and VIII, would also be depleted. Our material would not pass this test.

It was felt that tests had been devised according to preconceived ideas of which factor or factors might be responsible for the potential thrombogenicity. We sought the co-operation of in a critical examination of these tests and he presented this work in a paper at the Xth Congress of the International Society of Blood Transfusion, Helsinki, August 1975. No conclusion is yet possible concerning a test which would be applicable to all types of factor IX concentrate prepared for clinical use. We shall continue this work and in the meantime use our well-established simple recalcification time test and a general toxicity test in rabbits.

Factor VIII Standards: Following a decision (later reversed) that the NIBSC would no longer prepare a National Factor VIII Reference Preparation of freeze-dried plasma, and a request from the Directors of Haemophilia Centres at their meeting on 1st November, 1974, for assistance in this matter, it was agreed that Plasma Fractionation Laboratory, Oxford and Blood Products Laboratory, Elstree would jointly undertake to supply this need. Preliminary work to select a suitable buffer for preservation of the factor VIII was continued with the collaboration of the clinical laboratory of the Oxford Haemophilia Centre and later with of the NIBSC. This work will be the subject of a letter to the Editor of Thrombosis et Diathesis Haemorrhagica.

When the Director of NIBSC decided that that Institute would, after all, continue to prepare a National Factor VIII Reference Preparation, the material already prepared by BPL and PF Lab. was accepted by the Oxford Haemophilia Centre for use as a "house standard".

After discussions between and it was decided to express the potency of dried preparations of factor VIII in International Units, from 1.1.1975. This change will result in a reduction of the labelled value of the contents of a container of dried factor VIII concentrate by approximately 20 per cent. It will also be necessary to rewrite the recommended dose schedules to correspond.

pointed out that the amount of factor VIII per container prepared at PF Lab. and BPL was some 20 per cent more than is needed for routine treatment of minor bleeds if treatment is given at the first sign of need. Immediate treatment is one of the main advantages of home therapy. It was clear that there might be a notably more economic use of concentrate if the amount per container were reduced by 20 per cent. Consequently our previous practice of putting 350-400 units (as previously expressed (i.e. 1 unit is activity in 1 ml plasma) in each container was discontinued in favour of approximately 300 units (as previously expressed) or approximately 250 i.u. per container. The value in i.u. as determined by assay is given on the bottle label.

Factor IX Standard: A batch of factor IX type DE(1) was supplied to NIBSC for the preparation of a proposed International Standard for factor IX. The International Committee on Thrombosis and Haemostasis, meeting in Paris, July 1975, agreed that this standard should be recommended to WHO. It is expected that the adoption of the standard, as in

the case of factor VIII, will result in a reduction of the labelled value of the contents of a bottle of dried factor IX.

Clinical Trial of Factor IX: Work has continued towards a clinical trial of factor IX type DE(1) in patients in whom rapid or temporary reversal of anticoagulant therapy is necessary and in patients with liver disease undergoing liver biopsy. A document was prepared for use by the Medical Research Council when submitting its request for a clinical trial certificate to the Committee on Safety of Medicines. This document (copy attached) summarises experience in the use of factor IX type DE(1) in the treatment of liver disease during the five year period 1971-4.

Factor VII: It was noted that the yield of factor VII as a by-product of the preparation of factor IX type DE(1) had fallen to nearly zero for no apparent reason. Attempts have been made to improve the adsorption of factor VII on to DEAE-cellulose without success. Experiments have been carried out which confirm the possibility of recovering the factor VII from the supernatant of the DEAE-cellulose adsorption by adsorption on to DEAE-sephadex and subsequent elution with a strong salt solution followed by ultrafiltration. There are several practical reasons why this would not be the method of choice and we intend to continue the previous work to try to prepare this material in a reproducible form as a by-product of the factor-IX production.

Research on the degree of activation of the coagulation factors in the prothrombin group concentrates is a matter of great urgency. Following an unsuccessful attempt to differentiate positively between factors VII and VIIa by SDS electrophoresis, work is being done to assess the possibility of separating the two factors by molecular sieve, using the newly developed agarose/polyacrylamide gel matrix "Ultrogel" (Uriel, 1966 a and b, Uriel et al, 1971) available from LKB.

Refractometer: A simpler improved version of the refractometer used in the preparation of the DE(1) type factor IX (Dike, Bidwell and Rizza, 1972) has been built and shown to be satisfactory. The machine has a very simple optical system, a very high degree of stability, and is unaffected by the colour or turbidity of the solution. Fraction cutting points are electrically and audibly signalled, and the recorder outlet provides a 0-10 volt signal corresponding exactly to the analogue readout, obviating careful recorder calibration.

PRODUCTION

During 1974 the following were provided for clinical use:-

Factor VIII Concentrate: 4727 bottles, containing on average 375 units (1 unit = activity in 1 ml plasma), of factor VIII per bottle. The concentrate was supplied almost exclusively to the Oxford Haemophilia Centre.

Factor IX Concentrate: High potency type DE(1). 6569 bottles containing an average of 800 units (1 unit = activity in 1 ml plasma) of factor IX in 20 ml. Of these bottles, 728 were supplied to the Oxford Haemophilia Centre and 5841 to other hospitals. This total is an increase of 43.7 per cent over the amount supplied in 1973. Experience during the first half year of 1975 suggests that the demand

for the full year will be about 7000 bottles. As far as is known, sufficient concentrate is now prepared to treat all patients with congenital absence of factor IX.

Factor IX Concentrate type C: 77 Bottles containing material for reconstitution in 100 ml were supplied for the treatment of factor VII deficiency.

Note: The older 'type C' preparation contains factor VII; the high potency type DE(1) does not contain significant amounts of factor VII.

Staff

appointment will terminate at the end of August 1975. will be trained in the coagulation factor assay work and continue to take responsibility, under , as at present, for other parts of the control work (toxicity tests, arrangements for tests for sterility, HB Ag, agglutinins and biochemical and chemical determinations). See separate paper on arrangements proposed for coagulation assay work.

The vacancy created by has been filled, as from 1st October 1975, by the appointment of who has worked for the past (Curriculum vitae attached). will be responsible, under for the production of therapeutic fractions and will participate in research and development directed towards their improvement.

Authority is requested in retrospect for the appointment of a Technician, experienced in blood coagulation, from 1 September 1975. He will work in the coagulation assay laboratory. The man appointed was trained by and worked for five years in He then worked in . If his services are satisfactory, it is proposed to promote him to Senior Technician after a period of three months and approval for this is requested.

Authority is requested to appoint an additional junior technician in the assay laboratory from 1 July 1976 and an additional laboratory assistant from 1 April 1976.

Authority is also requested to upgrade an existing Technician appointment in the fractionation laboratory to Senior Technician in recognition of the responsible nature of the work (from 1 July 1976).

Accommodation

The flat roof above the fractionation laboratory mentioned in last year's report has continued to leak into the fractionation laboratory. Arrangements have now been made by the Oxford Area Health Authority (Teaching) to replace the roof in September 1975. The City of Oxford Fire Authority has strongly recommended that new safety measures should be installed.

The Drayton-Castle high vacuum high temperature steam steriliser still suffers repeated breakdowns.

The air conditioning authorised in the estimates for 1975-76 has been installed and is working satisfactorily. Members of the staff have spontaneously expressed their appreciation of the improved working conditions during this hot summer.

The -40°C deep freezes continue to break down. A representative from BOC Cryo Products - Edwards High Vacuum visited the laboratory and recommended that the existing cabinets were converted to cooling with liquid nitrogen. Our own calculation of the running costs (later confirmed by BOC) showed these would be prohibitively high.

A new electrical supply direct from a 11 KV sub-station has been installed to serve the laboratory. We understand that there is a proposal to instal new stand-by generating equipment to serve this area of the hospital. We have made it clear that we must retain our own independent generator, maintained by ourselves, which has proved entirely satisfactory.

As mentioned in the report for 1973, installation of the Drayton-Castle Autoclave led to a loss of one-third our storage space. The present situation includes use of part of the roof space for storage. This is dangerous because of fire hazard, difficulty of access and movement across unfloored rafters. The possibility of acquiring the temporary building (at present used as a library of the Haemophilia Centre) which will become available when building starts on the proposed extension to the clinical unit, seemed attractive but the problems of siting and associated costs would be expensive. Two buildings adjacent to the rear of the laboratory, which are at present used by other departments (one at the Radcliffe Infirmary for the storage of PM brains and the other as a store for general building items by the Works Department), would lend themselves admirably to upgrading. Exploratory discussions with Oxford AHA (Teaching) are in progress.

General

It is disappointing that our production of factor VIII is still limited by the supply of plasma.

8.8.75.