FACTOR VIII CONCENTRATE HIGHLY PURIFIED AND HEATED IN SOLUTION.

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The materials and methods in this paper are all standard techniques and sources.

The Results

The problem of producing a hepatitis-free Factor VIII concentrate has been solved by using two sequential steps. First of all there is a high enrichment of Factor VIII combined with a separation of some of the hepatitis virus followed by inactivation of the Dane particles not so separated by heating up the whole preparation in the presence of stabilising factors to protect the Factor VIII activity. This procedure is summed up in Table 1. This Table starts from a pooled cryo-precipitate and purification is achieved in five steps. First of all there is an aluminium hydroxide adsorption of the re-dissolved cryo-precipitate. The supernatant of the aluminium hydroxide adsorption is then further fractionated by a glycine precipitation. The supernatant of that precipitation is then further fractionated by a sodium chloride precipitation. This precipitate is redissolved in glycine and sucrose solution and incubated for 10 hours at 60°. The heated solution is then re-precipitated with sodium chloride to bring the Factor VIII out of the stabilising solution. The final step involves dialysis and the addition of albumin as a stabiliser and lyophilisation of the final product. The specific activity after step 5 before the addition of the albumin is 20 i.u./mg protein. This is equivalent to an enrichment of 1,500 fold over the starting plasma. The addition of 0.5% albumin is necessary to prevent the loss of activity in the protein solution during sterilisation by filtration and lyophilisation. To validate the purification procedure 1 ml of hepatitis infected serum was added to 1 litre of cryo-precipitate solution and the resulting pool subjected to the purification procedure. This starting material had a hepatitis antigen titre of 90 ng/ml and a chimpanzee infectious dose of 10^7. This was purified according to the procedure described and hepatitis antigen level measured at each step during fractionation using RIA. As can be seen from the left hand column of table 1 its antigen titre of 90 ng/ml was reduced to less than 1 ng/ml which was the limit of sensitivity of the test in use. Antigen concentration of 1 ng/ml is equal to 10^{-2.5} of the chimpanzee infectious dose units. To examine the necessity for the heating hepatitis infected step 1 litre of cryo-precipitate solution was processed as described but the heating step was omitted. Four monkeys were then injected with Factor VIII concentrate that had been prepared by a procedure including the heating step and four with material prepared using the procedure where the heating step was omitted. The result was significant and clear. All four control monkeys became infected with hepatitis whereas the others which received the heated Factor VIII concentrate showed
no symptoms of hepatitis B or of non A non B hepatitis. This result was verified by examination of the animals' tissues for the presence of hepatitis antigen. The results agreed with the infection results. The details of this experiment will be described in another paper.

The Factor VIII which had been prepared as described was characterised and compared with a commercial preparation by another manufacturer. The Behringwerke Factor VIII preparation described above had only one weak component in the β-globulin region by electrophoresis and this was identified by an immuno-fixation procedure as Factor VIII related antigen. The Factor VIII concentrate prepared without the heating step contained a small amount of fibronectin and a small amount of fibrinogen. These contaminating proteins together with a small amount of immunoglobulin are the main components of the commercial product prepared according to the method of Brinkhouse. The anti-haemophilic cryo-precipitate of Behringwerke contains the total spectrum of plasma protein and not surprisingly cannot be discriminated from total serum using this electro-phoretic technique.

Table 2 compares with the properties of Behringwerke Factor VIII concentrate and the commercial Factor VIII concentrate. All three concentrates have the same activity as units of Factor VIII per ml. However the heated Behringwerke preparation has the lowest protein concentration and therefore the highest specific activity in spite of the albumin which was added in a final concentration of 5mg/1 for stabilisation. The ratio of Factor VIII related antigen to Factor VIII clotting activity is 3.0 for both the Behringwerke preparation and 3.5 for the commercial preparation. There is no fibrinogen in the Behringwerke preparations and fibronectin is dramatically reduced. There is no immunoglobulin in the Behringwerke preparations.

The next section describes tests of the in vivo activity of the Factor VIII preparations in particular to examine the effect of the heat treatment on the molecular properties of the concentrate. Electrophoretic mobility and the in vivo half life and recovery were used as criteria for the comparison. Figure 3 shows the same region of a two dimensional immuno-electrophoresis for all three Factor VIII preparations. In vivo recovery and half-life of the Behringwerke preparation described are comparable with those of the unheated preparation and with data from the literature. Recovery from patients in which it was used prophylactically was about 70% and in cases of acute bleeding 60%. In the discussion it is said that the production method has been described to produce hepatitis safe Factor VIII concentrate which is based on two principles. High purification of Factor VIII with the simultaneous separation of hepatitis virus and subsequent inactivation of such hepatitis virus as is not separated in the first step by heating for 10 hours at 60°C.
in solution. The high purification is achieved with relatively mild conditions. The Factor VIII remains during the fractionation steps preferentially in solution whereas the insoluble contaminating protein such as fibrinogen become precipitated. For the heating step which is similar to the procedure used routinely for albumin stabilising factors are used. The use of glycine and sucrose is described for the first time as stabilising factors to protect Factor VIII and prevent its denaturation by heat. The heat treatment results in additional purification. Proteins which are not protected by glycine and sucrose become denatured in this step and fall out of solution. From the supernatant of the heated step the Factor VIII activity is separated by salt precipitation and comes out of the solution as a native product as can be seen from the analyses in Table 2, Table 3 and Figure 3. The data presented in these analyses agree with the enrichment factor of 1,500 compared with a starting plasma. The results of tests using animals show that the hepatitis virus deliberately added to the starting plasma in some of the preparations was definitely separated from the Factor VIII during the preparation of the concentrate. The final product is not infective in the chimpanzees. The same result has been found in the first clinical studies which are reported in reference 16.

Points of importance to note about this paper are that salt concentrations either for the precipitation or for the buffers are not given and that there is no mention of the yields of Factor VIII either overall or at intermediate stages in the preparation the concentrate.