FACTOR VIII. PROGRESS IN FRACTIONATION

RESEARCH AND DEVELOPMENT

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PETER R. FOSTER
PROBLEMS IN THE MANUFACTURE
OF FACTOR VIII CONCENTRATES

1. QUANTITY (YIELD)

OBJECTIVE - SELF SUFFICIENCY

2. QUALITY

OBJECTIVE - HIGHER POTENCY

LESS FIBRINOGEN

NON-INFECTION
P.F.C. PROCESS FOR FVIII PREPARATION

1973 - 1983

<table>
<thead>
<tr>
<th>STAGE</th>
<th>PROCESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FRESH FROZEN PLASMA</td>
</tr>
<tr>
<td>2</td>
<td>RECOVER CRYOPRECIPITATE</td>
</tr>
<tr>
<td>2</td>
<td>PREPARE CRYO. EXTRACT</td>
</tr>
<tr>
<td>3</td>
<td>ADSORB IMPURITIES</td>
</tr>
<tr>
<td>3</td>
<td>CENTRIFUGE &amp; FILTER</td>
</tr>
<tr>
<td>3</td>
<td>STABILISE</td>
</tr>
<tr>
<td>4</td>
<td>FILTER TO 0.2 µM</td>
</tr>
<tr>
<td>4</td>
<td>FREEZE DRY</td>
</tr>
<tr>
<td>4</td>
<td>FVIII CONCENTRATE</td>
</tr>
</tbody>
</table>

(INTERMEDIATE PURITY)
FVIII YIELD AT EACH STAGE
IN THE PRODUCTION PROCESS

<table>
<thead>
<tr>
<th>STAGE</th>
<th>% FVIII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1978</td>
</tr>
<tr>
<td>1. FRESH FROZEN PLASMA (6 hr)</td>
<td>100</td>
</tr>
<tr>
<td>2. CRYOPRECIPITATE:EXTRACT</td>
<td>37.2</td>
</tr>
<tr>
<td>3. ADSORBED EXTRACT</td>
<td>32.0</td>
</tr>
<tr>
<td>4. FREEZE DRIED PRODUCT</td>
<td>25.0</td>
</tr>
</tbody>
</table>

REFERENCES

(2) FOSTER et al Vox Sang. 42, 180 - 189, 1982
METAL ION PRECIPITATION

FRESH FROZEN PLASMA

\[ \downarrow \]

RECOVER CRYOPRECIPITATE

\[ \downarrow \]

TRIS \[ \rightarrow \] CRYOPRECIPITATE EXTRACT

\[ \downarrow \]

ZINC/HEPARIN \[ \rightarrow \] PRECIPITATION

\[ \downarrow \]

SOLUTION

\[ \rightarrow \]

SOLIDS

FIBRINOGEN (75%)

FIBRONECTIN (65%)

\[ \rightarrow \]

FVIII (90%)

VWF (90%)
MEASUREMENT OF IONISED CALCIUM IN FACTOR VII SOLUTIONS

Ionised Calcium (-mV)

SODIUM CITRATE (mM/1)

CALCIUM CHLORIDE (mM/1)
THE EFFECT OF CITRATE

1. DISADVANTAGE
   1.1 LOSS OF FACTOR VIII ACTIVITY

2. ADVANTAGES
   2.1 ANTIMICROBIAL EFFECT
   2.2 IMPROVES FILTRATION (0.2 µM)
   2.3 IMPROVES PRODUCT SOLUBILITY
   2.4 CONTRIBUTES TO OSMOLARITY
   2.5 PREVENTS SECONDARY PRECIPITATION DURING ZINC PROCESS.
FVIII STABILITY IN CRYOPRECIPITATE EXTRACT
FOLLOWING A1(OH)3 ADSORPTION

Before Citrate Addition

After Citrate Addition

FVIII:C (%) Mean & SD (n = 15)

Time (Hours)

0 1 2 3

(p < 0.025)

(p < 0.001)
# RECOVERY OF FVIII:C AT DIFFERENT CONCENTRATIONS OF IONISED CALCIUM

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>CITRATE ADDED (mM)</th>
<th>N</th>
<th>FACTOR VIII:C (%)</th>
<th>MEAN ± S.D.</th>
<th>INCREASE (%)</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FROZEN</td>
<td></td>
<td></td>
<td></td>
<td>WITHOUT ADDED CALCIUM</td>
<td>WITH ADDED CALCIUM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25</td>
<td>100</td>
<td>-</td>
<td>15.4</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>84.4 ± 12.1</td>
<td>97.4 ± 14.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREEZE DRIED</td>
<td>20</td>
<td>25</td>
<td>96.5 ± 29.0</td>
<td>128.2 ± 25.1</td>
<td>32.8</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

## NOTES

1. ALL SAMPLES HELD FOR 2 HOURS AT R.T. THEN FROZEN.
2. FVIII:C ASSAYS BY 1-STAGE METHOD.
## FACTOR VIII INFECTIVITY

### 1. PROBLEMS

1.1 HEPATITIS B

   HEPATITIS NANB

1.2 OTHER (CMV, AIDS)

### 2. PASTEURISATION (60°C, 10 HR)

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>STABILISER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 ALBUMIN</td>
<td>CAPRYLATE</td>
<td>GELLIS et al. J. Clin. Invest. 27, 239-244, 1948</td>
</tr>
<tr>
<td>2.2 AT III</td>
<td>CITRATE</td>
<td>HOLLEMAN et al. Thromb. Haem. 38, 201, 1977</td>
</tr>
<tr>
<td>2.3 FVIII</td>
<td>SUGAR/GLYCINE</td>
<td>SCHWINN et al. German Patent 291671: 1979</td>
</tr>
<tr>
<td>(8% YIELD)</td>
<td></td>
<td>(HEIMBURGER et al. Haemost. 10 (S1), 204, 1981)</td>
</tr>
</tbody>
</table>
R & D PROJECTS INTEGRATED TO
FORM NEW FVIII PROCESS

1. CONTINUOUS PLASMA THAWING.

2. ZINC FRACTIONATION.

3. CONTROL OF IONISED CALCIUM.

4. HEAT TREATMENT WITH IMPROVED STABILISERS.