TSG

P.F.C. RESEARCH & DEVELOPMENT DEPARTMENT

A SUMMARY REPORT

P.R. Foster, April 1975

Contents

A. Staffing

Staff	Grade
P. R. Foster	Senior Biochemist
C. Turnbull	Senior Biochemist
M. R. Patterson	Senior Biochemist
S. Middleton	Senior Biochemist
D. A. Ross	Basic Grade Physicist
I. Dickson	Technician
A. Fairbairn	Laboratory Assistant

B. Activities

1. Cohn Fractionation

1.1 Development of Computer Controlled Fractionation

Encompasses the final stages in the design and development programme to produce a basic continuous fractionation unit with semi-automatic computer control. Includes the C.S.V.M. commissioning programme at the new Centre.

Importance: Will give processing capability of at least 2000 litres plasma per week (for PPS) using modular units.

Participants: P. Foster, A. Ross.
Engineering staff (B. White, C. Watson)
Process Staff - since 1975 (A. Dickson, J. Fitchie, J. Dyer, J. Blundell).

Time to complete (approx): 6 months (commenced 1973).

1.2 C.S.V.M. - Further Development and Evaluation

Extension of continuous processing to other fractionation schemes including re-work. In-depth study and evaluation to fully define capabilities of C.S.V.M. Re-design and/or improvement of individual operations if necessary. Will involve laboratory and in-process studies.

Importance: Will extend C.S.V.M. fractionation to the maximum number of P.F.C. products. Will allow process optimisation in terms of yield, purity and daily work schedules. Will allow substantial increase in C.S.V.M. throughput.

Participants: P. Foster, I. Dickson.
Engineering staff (B. White, C. Watson).

Time to complete: 3 years (commenced 1974).

1.3 Precipitate Conditioning for centrifugal separation

Precipitate conditioning has always formed an important part of fractionation. However, little information has been published and the physico-chemical changes involved have not been identified.

The use of sonic vibrations for this purpose is being studied, as well as the importance of mixing and equilibration in the conventional batch aging period.

Importance: Will allow improved centrifugal separation, particularly in continuous processing. Will lead to a re-evaluation of solid-liquid separation and will contribute to the knowledge of plasma fractionation and protein isolation in general.

Participants: P. Foster, A. Ross, I. Dickson.

Time to complete: 3-4 years (commenced 1974).

2. Coagulation

2.1 Preparation of a factor IX concentrate with reduced HBAg activity

Involves optimisation of factor IX recovery and removal of HBAg from D.E. F. IX by manipulation of pH, protein concentration, ionic strength and PEG concentration. Includes development to production scale.

Importance: Will replace D.E. F. IX with a concentrate 3-5 times more potent, and with a reduced HBAg content.

Participants: J. K. Smith, S. Middleton, external.

Time to complete: 18 months (approx.) (Commenced 1971).

2.2 Four-factor concentrate containing factor IX

Modification of method of N.R.C. with particular emphasis on F. VII recovery and improved hygiene.

Clinical needs are uncertain and HBAg contamination and thrombogenicity are potential problems.

Importance: Would replace PPSB with a product like D.E. F. IX but containing clinically useful amounts of factor VII.

Participants: J. K. Smith, S. Middleton.

Time to complete: Suspended. (commenced 1971).

2.3 Preparation of antithrombin III by affinity chromatography

Development of adsorption techniques to allow the isolation of antithrombin III from F. IV pastes presently discarded. Also involves development of analytical techniques.

Importance: For possible use in anticoagulant therapy or as an additive to coagulation factor products for control of thrombogenicity.

Would also allow considerable experience in the application of a very promising technique, possibly relevant later to factor VIII purification.

Participants: J. K. Smith, S. Middleton.

Time to complete: Only intermittent at present (commenced 1974).

2.4 Thrombogenicity of factor IX - containing concentrates

Continued co-operation with other laboratories, including Edinburgh BTS, in assessing potential in vivo thrombogenicity by in vitro tests.

Importance: Improvement of current quality control of D.E. F. IX and PPSB, and possible influence on projects 2.1 and 2.2.

Participants: J. K. Smith, S. Middleton, QC staff as necessary, external collaborators.

Time to complete: 2 years (commenced 1974).

3. Immunoglobulins

3.1 Preparation of highly potent therapeutic anti-D IgG

Development of DEAE-sephadex method on approximately 2 litre scale.

Importance: Preparation of anti-D IgG at about 1000 µg/ml, suitable for intravenous use.

Participants: J. K. Smith, M. Patterson.

Time to complete: Suspended. (commenced 1973).

3.2 Preparation of intravenous IgG, including standardisation of tests of anti-complementary activity

Study of "depolymerisation" of normal IgG from conventional fraction II. Particular interest in the use of pepsin and acid hydrolysis.

Importance: Expected to lead to preparation of 5% normal IgG in 50-100 ml doses, for passive protection e.g. of hypogammaglobulinaemic patients, or treatment of septicaemias.

May allow technical capability to make all hyperimmune IgG fit for i.v. use, doubling its effectiveness.

Participants: J. K. Smith, M. Patterson.

Time to complete: 2 years (commenced 1972).

3.a Plasminogen, IgM and IgA from Fraction III

Study of isolation methods, e.g. euglobulin precipitation, PEG precipitation, gel filtration. These approaches probably supplanted by new methodologies; also problems concerning HBAg content of Fraction III.

Importance: Recovery of potentially therapeutic proteins from waste fraction.

Participants: J. K. Smith.

Time to complete: Suspended (commenced 1970).

4. Albumin

4.1 Preparation of Albumin Solutions by Forced Flow Electrophoresis

A study of the electrophoretic separation of albumin from α and β globulins in a supernatant II + III solution. Design of a unit for integration into the continuous fractionation scheme.

Importance: Expected to increase the yield and purity of albumin solutions. Will also provide fundamental knowledge for the general application of electrophoresis on the process scale.

Participants: J. Watt, P. Foster, C. Turnbull.

Time to complete: 3 years (commenced 1971).

4.2 Removal of ethanol from albumin by gel filtration

Development of gel filtration technique involving study of gel materials and operating conditions on laboratory and pilot scale.

Requires further development to process scale.

Importance: Expected to supplement or replace vacuum distillation for removal of ethanol for PPS.

Also potential use for removal of low molecular weight reagents from other protein concentrates.

Possible relevance to removal of "bradykinin" from albumin (5.2).

Participants: J. K. Smith, A. Dickson.

Time to complete: Suspended. (commenced 1970).

4.3 Preparation of Salt-Poor Albumin from Standard FIV_4 + V or F V + VI, without ethanol re-work

Study to extend gel filtration or adsorption techniques to albumin purification.

Importance: Replace Cohn re-work with more efficient and cheaper process.

Participants: J. K. Smith, A. Dickson, T. McQuillan.

Time to complete: Suspended. (Commenced 1972).

5. Other

5.1 Particle analysis for routine Quality Control

Initial evaluation of the Prototron and the Coulter Counter as particle monitoring instruments.

Further evaluation of the Coulter Counter technique to define capability and allow routine use.

Particularly concerned with both routine and non-routine analysis of crystalloid products.

Also interested in the application of the instrument to other research activities.

Importance: Will allow analysis as recommended in the British Pharmacopoeia.

Participants: P. Foster, I. Dickson.

Time to complete: 6 months (commenced 1974).

5.2 A preliminary study of Kinin-like activity of P.F.C. products

Measurement of bradykinin-like activity of P.F.C. products and identification of the source of any activity. Assessment of the importance of such activity vis-a-vis reduction in blood pressure of patients following infusion of PPS.

Importance: Will define limits for clinical use of present PPS stock and will provide information to determine the necessity for a more detailed study.

Participants: J. K. Smith, P. Foster, external.

Time to complete: 6 months (commenced 1975).

5.3 Problems of Factor VIII recovery

Evaluation and re-design of systems and processes leading to the production of Factor VIII as an intermediate concentrate. Includes study of plasma input from RTC's, plasma freezing and plasma thawing.

Importance: Expected to lead to a substantial increase in recovery of Factor VIII from F.F.P.

Participants: J. Watt, P. Foster.

Time to complete: probably 1 year (commenced 1974).

5.4 Computer Application Studies

Study and preparation to allow maximal P.F.C. benefit from the computer facility.

Includes: design and improvement of programs for data handling and data storage for process, research and quality control functions; extension of computer for information handling and storage; extension of computer monitoring to manual process operations; general modifications and development of process control systems.

Importance: essential for efficient operation of whole P.F.C. facility.

Participants: P. Foster, A. Ross.

Time to complete: indefinite (commenced 1973).

5.5 Process optimisation studies

Mainly involves multiple investigations concerning specific process operations requiring evaluation or re-design; also general "trouble-shooting".

Includes: centrifuge studies,
physico-chemical properties of solutions,
problems of fluid transport,
problems of mixing, agitation,
and heat transfer.

Participants: P. Foster, I. Dickson, and others.

Time to complete: indefinite (commenced 1973).

5.6 Recovery of C 1s esterase and its inhibitor from plasma

Development of known technologies for inexpensive recovery of these proteins without significant loss of other valuable proteins.

Importance: Primarily for structural studies, but inhibitor of possible therapeutic value in congenital deficiency states.

Participants: J. K. Smith, M. Patterson, QC Staff, Production staff, external.

Time to complete: 6 months.

C. Recent Reports/Further Information

1. Cohn Fractionation

1.1 Foster, P.R. (Jan. 1975) internal report

"An interim report on the status of C.S.V.M. fractionation".

Foster, P.R. (Feb. 1975) internal memorandum

"C.S.V.M. Commissioning Programme"

Minutes of C.S.V.M. weekly meetings.

1.2 See 1.1

1.3 Foster, P.R. (Feb. 1975) report of the P.F.C. Seminar, Nov. 1974.

"The use of sonic vibrations for the conditioning of protein precipitates prior to centrifugal separation".

2. Coagulation

2.1 Middleton, S. (Oct. 1974) internal report.

"PEG Fractionation of D.E. F. IX eluate".

2.2 Johnson, A.J., Newman, J., Semar, M., Middleton, S. and Smith, J.K. (1973).

"Removal of Hepatitis-B Antigen (HBAg) from coagulation factor II, VII, IX and X concentrates for clinical use".

presented at the Congress of The International Society of Thrombosis and Haemostasis, Vienna, Austria.

2.4 Smith, J.K. (Oct. 1974).

Report of ICTH Meeting & Working Parties on safety of factor IX concentrates, Basle October 1974.

3. Immunoglobulins

3.1) Patterson, M.R. (Aug. 1974) internal report
3.2) "Status of R & D projects to extend the
~~3.3)~~ therapeutic value of human IgG".

4. Albumin

4.1 Turnbull, C. (Dec. 1974) internal report

"Biers Machine Operations, period June - November 1974".

4.2 Dickson, A.J. and Smith J.K. (1975)

"Alternatives to freeze-drying for the removal of ethanol from plasma proteins. II Gel filtration of albumin".

Vox Sang. 28, 90-100.

5. Other

5.1 Foster, P.R. (July 1974) internal report

"The Prototron for P.F.C. Quality Control and Research".

Foster, P.R. (Aug. 1974) internal report

"The Coulter Counter for P.F.C. Quality Control and Research".

5.2 Smith, J.K. (March 1975) internal memorandum.

"Interim report on "Bradykinin" in PPS".

5.3 Watt, J.G. and Foster, P.R. (Feb. 1975) internal report

"Polyethylene Plasma Transfer Packs and Plasma Quality".

5.4 Ross, D.A. (March 1975) departmental memorandum.

"Activities in the period October 1974 - March 1975".

5.5 Foster, P.R. (Aug. 1974) internal report.

"Diffusivity data for process design".

D. Some Potential Activities (1975)

1. Evaluation and optimisation of cold ethanol fractionation. (MP, CT)
2. Use of polyelectrolytes in fractionation. (S.M.)
3. Fibrinogen removal, in F VIII preparation, using immobilised dextran sulphate. (S.M.)
4. Preparation of plasminogen by affinity chromatography. (S.M.)
5. Further use of polymers in fractionation. (S.M.)
6. Preparation of complement components. (M.P.)
7. Preparation of limulus lysate for routine use in quality control. (M.P.)
8. Development of batch processing of small pool fractions. (S.M.)
9. Reactivation of project 4.2. (C.T.)