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National Blood Transfusion Service

PROTEIN FRACTIONATION TECHNOLOGY WORKING PARTY REPORT 1981

1.1. To meet internationally accepted requirements for plasma proteins for the National Health Service, production in England and Wales must be raised to fractionate 450,000 litres* of plasma a year.

1.2. The chain of technical actions required prior to the building of the new plant is considerable. It is essential that a ministerial decision be made at the earliest possible date which would permit employment of a plant engineer to coordinate planning and a specialist architect. Only by such action can the cost information required for subsequent government decisions be provided at a rate consistent with the urgent need to establish an enlarged working operation which conforms to the requirements of Medicines Division.

1.3. Production plans rest upon a very substantial increase in plasma supply and an assurance of consistent quality. Coordination of this supply is recognized by the Working Party to be a key factor. Keeping the increasing plasma volumes in step with the growing capacity at BPL Elstree will be a most delicate manoeuvre.

TECHNICAL RECOMMENDATIONS

2.1. A brief summary of plasma fractionation methods at BPL Elstree is presented in Appendix 1, together with an indication of the present and proposed scales of production which are determined by the demand for albumin and anti-haemophilic factor (VIII).

2.2. The Working Party concluded that plasma fractionation should continue to be centred on cold ethanol precipitation (Appendix 2). This is presently preceded by the low temperature precipitation of anti-haemophilic factor (VIII) from the 50% of plasma which is available in a fresh frozen state (FFP). In future plasma will be required primarily in this form permitting a maximum recovery of factor VIII. The Working Party received written and verbal evidence from the principal producer of chromographic systems which represent a potential alternative but was satisfied that these are not yet appropriate other than as noted in 2.7.

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*This figure is subject to alteration by the Advisory Committee on the National Blood Transfusion Service but seems likely to be a reasonable estimate.

2.3. The different plasma-ethanol mixing technologies at PFC Liberton and BPL Elstree are a small element of the total process but have some impact on the process layout and operation. Information is only just coming to hand which may eventually permit useful integration of the different approaches. The proposed experimental trials of fully continuous (14 x 24 hr) operation at Liberton are important in this regard and as they illuminate manning problems: they should be given every encouragement. In the meantime, the Working Party has established a scheme within which some flexibility is maintained and which balances certainty of start-up against a small additional cost (Appendix 2). The Working Party received evidence from the centrifuge manufacturer most active in adapting machines for better performance of this critical step and took note of developments now occurring.

2.4. The preparation of detailed architectural plans for housing such a complex process in special working environments is a matter for discussion between a full-time architect, transfusion staff and Medicines Division. The Working Party limited its discussion to issues such as the timetable which would be needed for construction and to some of the principal constraints (Appendix 3). It concluded that the economic advantages to the nation indicated in an earlier memorandum from Mr D. Smart and broadly confirmed by DHSS analysis would justify adoption of special contractural procedures for which there are precedents in government departments. The time-scales indicated in Appendix 3 would be altered by any changed assumptions about the final desired scale of operation but the impact would be small.

2.5. Some of the factors which bear upon the balance of good pharmaceutical practice and economic construction and operation of a new plant have been defined (Appendix 4). However, a full brief prepared for the BPL director by the plant engineer in conjunction with Medicines Division will be required at the earliest possible date by the site architect.

2.6. The Working Party discussed in detail the suitability of the Elstree site. It concluded that the geographical area is excellent in terms of supply of plasma and provision of fractions. The site does have some problems and further liaison with local and

regional planning authorities now seems sensible. However, similar problems are likely to occur elsewhere and, given the urgency of the rebuilding and the need to maintain existing production, the Elstree site seems appropriate. Enlargement of the facility in this apparently rural area would be consistent with the presence in the immediate locality of large works. Outstanding scientific establishments are situated nearby and, with minimal allocation of travel resources, staff can maintain the vital stimulus needed to sustain scientific excellence at BPL.

2.7. Newer technologies involving adsorbents and other precipitants are useful for the recovery of trace components and can be accommodated within the framework of planning for fractional precipitation (Appendix 5). Given the time taken to accomplish rebuilding, it will be essential constantly to re-examine the extent to which such new approaches will be able to replace ethanol fractionation and the consequences for plant layout and services. This will be a key role of the research department. Potential improvements in product yield, which are already evident in laboratory studies, should be vigorously pursued.

For anything other than laboratory-scale trials the quantity of human plasma needed and its intrinsic value demands that final products are utilized for therapy. Therefore pilot and pre-production trials must be to the same rigorous standards of fractionation technology and quality control as normal production. A new facility must take account of this requirement and for the need not to disturb production during such trials. In practice this means that pilot facilities meeting Medicines Division requirements must be an integral part of a new facility.

2.8. Modern data acquisition and handling techniques can be applied to the fractionation process and to stock and quality control. They should be made compatible with automated data records from the regional centres. Both can be consistent with the requirements of Medicines Division for hard copy reports (Appendix 6).

2.9. The Working Party has examined the issues involved in raising plasma supply and its quality. In particular, they have considered the demands made on BPL in the transitional period leading up to the new full level of production (Appendix 7). It will be necessary to

re-appraise this matter at regular intervals in conjunction with the Advisory Committee for the National Blood Transfusion Service.

2.10. The Working Party noted that more stringent safety and regulatory requirements and the greater size and complexity of the operation will demand specialist staff in fields such as personnel training and management and in data processing.

2.11. New scientific developments, particularly in tissue culture and genetic engineering, have been evaluated (Appendix 8). Given speedy development of a new facility, the return on investment will have occurred before the products of such innovations can have been cleared for general use. However, the Working Party believes that the Blood Products Laboratory should be a party to discussions with other DHSS agencies such as CAMR Porton and possibly with the NEB concerning developments which rely heavily on expertise in evaluating plasma protein quality and on the use of human plasma waste products.

2.12. Reagents for the evaluation of plasma quality are an essential internal requirement for production at BPL and their availability to other Health Service agencies has brought substantial savings. The production of reagents and specialist materials from otherwise waste fractions represents both a full utilization of plasma resources and manpower at BPL and a foundation of scientific excellence necessary for a high technology centre.

2.13. Efficient implementations of the technical recommendations will be governed largely by the organizational framework within which the operation is undertaken. A framework within the Health Service which permits efficient functioning of the new advanced manufacturing facility will be essential to its success.

Appendix 1 Present plasma fractionation schemes with present and proposed scales of production

The human plasma proteins, prepared for clinical use either by the Blood Products Laboratory (BPL), Elstree, or by the Plasma Fractionation Laboratory (PFL), Oxford, fall into three groups related to their biological properties. They are:-

(a) Coagulation Factors

including Factor VIII (Anti-haemophilic Factor AHF), Factor IX complex (Factors II, IX and X), Factor VII, Fibrinogen.

(b) Immunoglobulins

including normal Immunoglobulin and specific Immunoglobulins, e.g. anti-Rh(D) and anti-Tetanus.

(c) Albumin preparations

including Plasma Protein Fraction (PPF), Salt-poor Albumin, Albumin solution in physiological strength saline.

Future production in a new laboratory may be expected to include this range of preparations, especially Factor VIII, Factor IX complex, normal and specific immunoglobulins, PPF and Albumin, together with others which at the moment constitute either a minor production commitment or which are still in development.

The main objective is to recover all useful material from the available source material. In determining the methods of fractionation to be used, the clinical needs for the various protein fractions must be related to the quantity of source material available, and to the feasible purity and clinical efficacy of the product, together with other factors such as stability and possible side-effects caused by impurities. The preparation of plasma proteins at Elstree and Oxford follows a well-defined series of separations, the sequence of which is governed by:

(i) the nature of the protein; the more labile being removed first

(ii) the nature of the technique required for each separation; the methods employed in the early stages having to be compatible with those which follow later.

The diagram shown overleaf summarizes the main points in the sequence of separations.

The first group to be isolated are the COAGULATION FACTORS. These factors are prepared from FRESH FROZEN PLASMA, provided by the Regional

Transfusion Service of England and Wales. The present scale of operation involves the fractionation of approximately 70,000 litres of fresh frozen plasma per annum. The projected annual demand for Factor VIII (90 million i.u.) is such that production capacity in a new laboratory would need to be increased to 450,000 litres per annum, estimated in current yields.

Factor VIII is the first to be separated by controlled thawing of the fresh frozen plasma, a process called CRYOPRECIPITATION.

After the cryoprecipitation stage has removed the FACTOR VIII from the plasma, the residual supernatant called CRYOPRECIPITATE SUPERNATANT is used for the preparation of FACTOR IX complex, which is removed chromatographically by DEAE-CELLULOSE ADSORPTION. At present, approximately 60% of the starting fresh frozen plasma is used as a source of this factor.

Next in sequence, FACTOR VII is prepared from the Factor IX supernatant by a further chromatographic treatment, DEAE-SEPHAROSE ADSORPTION. Clinical demand is such that only 1200 litres of the starting fresh frozen plasma is used in preparing this fraction.

Other clotting factors, such as FIBRINOGEN and THROMBIN represent a minor production commitment.

After the separation of the Coagulation Factors has been completed, the residual supernatants (COAGULATION FACTORS SUPERNATANT, CFS) are of value for their albumin and immunoglobulin content. Fortunately, the procedures used for the separation of the Coagulation Factors do not alter significantly the state of these supernatants from that of plasma, so that the method used for the production of albumin and immunoglobulin from whole plasma can also be used on the CFS.

Albumin and immunoglobulins are obtained by precipitation using a method called COLD ETHANOL FRACTIONATION (CEF). The starting material for this process may be CFS or TIME-EXPIRED PLASMA (TEP), and is usually a mixture of both. TEP is plasma separated from whole blood which has passed its expiry date. Although it is unsuitable for the preparation of labile factors such as Factor VIII, etc., it is a valuable source of albumin, immunoglobulins and other products.

The present scale of operation involves the fractionation of approximately 60,000 litres of TEP per annum, together with the CFS derived from 70,000

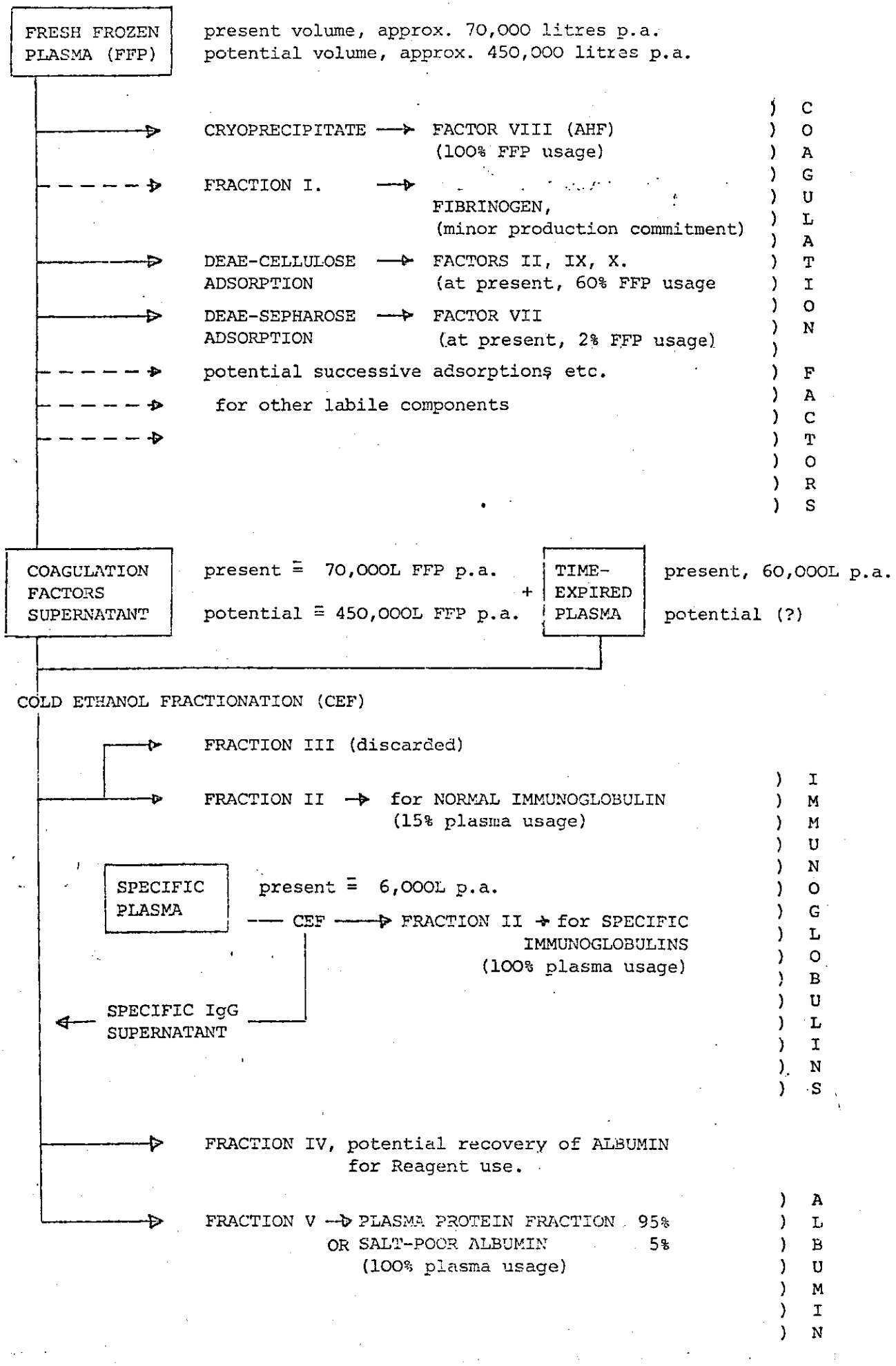
litres of fresh frozen plasma.

The separation by CEF of albumin and immunoglobulins, together with other plasma proteins, is achieved by the selective adjustment of variables, such as ethanol concentration, pH and temperature. The protein fractions are isolated stepwise in a series of precipitations. Precipitates are removed from suspension by centrifugation leaving a supernatant which is taken for the next stage of precipitation. The immunoglobulins are contained in FRACTION II and albumin in FRACTION V, using the terminology of Cohn, who developed ethanol fractionation.

Immunoglobulin prepared from TEP and CFS is called NORMAL IMMUNOGLOBULIN and contains a broad spectrum of the IgG antibodies which are present in the plasma of blood donors in England and Wales. Normal immunoglobulin prepared from plasma obtained from other parts of the world may differ in the specificity and titre of the antibodies it contains.

The SPECIFIC IMMUNOGLOBULINS (e.g. anti-Rh(D) and anti-Tetanus, etc.) are prepared by CEF from donors or from pools of plasma, specially selected for their specific antibody content. Approximately 6000 litres of specific plasma are fractionated per annum. Having removed the specific immunoglobulin from the plasma, the supernatant is then added to supernatants for recovery of albumin.

The remainder of the plasma proteins are contained in Fractions III and IV. A small but significant quantity of albumin is contained in Fraction IV and development work is in progress to recover this.


FRESH FROZEN PLASMA (FFP)
present volume, approx. 70,000 litres p.a.
potential volume, approx. 450,000 litres p.a.
CRYOPRECIPITATE → FACTOR VIII (AHF) (100% FFP usage)
FRACTION I. → FIBRINOGEN, (minor production commitment)
DEAE-CELLULOSE ADSORPTION → FACTORS II, IX, X. (at present, 60% FFP usage
DEAE-SEPHAROSE ADSORPTION → FACTOR VII (at present, 2% FFP usage)
potential successive adsorptions etc.
for other labile components
COAGULATION FACTORS SUPERNATANT
present ≡ 70,000L FFP p.a.
potential ≡ 450,000L FFP p.a.
+
TIME-EXPIRED PLASMA
present, 60,000L p.a.
potential (?)
COLD ETHANOL FRACTIONATION (CEF)
FRACTION III (discarded)
FRACTION II → for NORMAL IMMUNOGLOBULIN (15% plasma usage)
SPECIFIC PLASMA
present ≡ 6,000L p.a.
CEF → FRACTION II → for SPECIFIC IMMUNOGLOBULINS (100% plasma usage)
SPECIFIC IgG SUPERNATANT
FRACTION IV, potential recovery of ALBUMIN for Reagent use.
FRACTION V → PLASMA PROTEIN FRACTION 95%
OR SALT-POOR ALBUMIN 5%
(100% plasma usage)
COAGULATION FACTORS
IMMUNOGLOBULINS
ALBUMIN

Appendix 2 Fractionation Technology

Decisions on plasma fractionation must be made at two levels: the kind of chemistry to be used and the process engineering by which the chemistry is operated at a production scale.

The Working Party discussed the alternatives to cryoprecipitation and ethanol fractionation and received detailed evidence on chromatography. The members concluded that at present the conventional methods are preferable for the main fractionation. Precipitation techniques have the capacity to handle the large volumes needed and the use of ethanol inhibits microbial contamination. Chromatographic fractionation of proteins is intrinsically a slow process and while well suited to ancillary fractionation is not protected from contamination in the same way. The Working Party's conclusion is supported by the opinions and actions of other countries and, for example, new facilities in Finland and New York, USA, have adopted established precipitation techniques.

The choices with respect to the process engineering are more difficult to make. Two related decisions in particular will have a considerable impact upon the building of a new production facility. They concern the number of shifts to be operated per 24 hours and whether fractionation will proceed batchwise or semi-continuously.

The UK should be in a uniquely strong position to assess semi-continuous operation versus conventional batch operation since the former has been pioneered at the Edinburgh Protein Fractionation Centre. Sadly, lack of overall planning in the UK has meant that this facility has never operated for more than a single shift per 24 hours. In a continuous precipitation process where the plasma protein and ethanol are mixed continuously and immediately centrifuged it is important to minimize the start-up and shut-down periods where the process is not in steady-state. In single shift

operation such periods make up a significant proportion of working time. In spite of this limitation, the semi-continuous process has given good results in the hands of a staff well versed in process engineering. The staff of the Elstree facility are not as process engineering-orientated and could only be expected to approach the effectiveness of the originators of the process slowly unless a very direct technical linkage was arranged immediately. Though very short trials of day and night operation are planned at Liberton they can only give preliminary information and routine operation of a 24-hour fractionation day seems likely to be established too late to provide data for decisions at Elstree. There are considerable areas of scientific and technical ignorance about precipitation of proteins and about centrifugation which are reflected in potential weaknesses in continuous fractionation. For example, precipitates are more readily separated after ageing and batch processes can be timed to permit this. Recent techniques to speed the process are promising but not yet defined adequately. As a consequence, centrifugation of batch aged material appears to be more effective, though direct comparisons are not easy. This leads to the strategic question of whether the UK should base its plasma fractionation entirely upon semi-continuous operation which may well prove very efficient but for which there is yet not full information.

If the new Elstree facility were to be operated 24 hours a day on a three shift basis, the arguments for continuous operation would be at their strongest and, given successful operation, there would be a significant saving in capital expenditure. However, it is uncertain whether this is feasible. It involves agreement on pay structures which can be operated within the Health Service and, perhaps just as important, the relative attractiveness of employment at Elstree versus other local companies. Past experience suggests some difficulty: the work is demanding and as safety standards

are raised the conditions will be more constraining; the site is rather isolated even though access by car is good.

If adoption of two shift batch operation is contemplated it seems important to consider what flexibility would subsequently exist, given that the date of entering production could be 6 - 7 years away.

Discussion with Medicines Division make it clear that any physical alterations to a process will entail shut-down of the whole process area. It is also evident that production scale trials can only be undertaken in an area of environmental standard as high as that of routine production since the product is so precious that it will be used clinically. Finally, the nature of the centrifuges used means that process streams must be split and the scale of batch mixing vessels without sub-division will put very large volumes of plasma at risk at any one time.

The above considerations suggest that a twin precipitation facility would be appropriate. Given the low temperature environments needed, both process areas would have to be substantially constructed during initial building but the second stream might not be fully developed initially. Construction and particularly the method of providing services should allow for innovation. This might take the form of continuous operation or the introduction of a greater chromatographic/adsorption separation element. The disproportionate size of batch mixing tanks versus the rest of the plant and the small tanks needed for continuous operation suggests that batch tanks should either be kept small by using multiples or sited in such a way that they could be removed at a future date.

A switch from two shifts per day batch operation to three shifts per day semi-continuous processing could in principle permit a substantial increase in throughput. Thus it might be appropriate to set an initial production target in the centre of the range of projected possible require-

ments and use the ability to raise production to satisfy extra demand at a later date. If this approach is contemplated its consequences for other sectors of the facility will have to be explored in more detail.

The suggested approach is a compromise and one which need not have been necessary. It is essential for the future efficiency of UK plasma fractionation that there is better technical coordination of the Edinburgh and Elstree facilities. Many areas such as centrifugation, process control and data handling, solvent recovery and architectural planning with respect to the new plant at Elstree and extensions at Liberton should provide the basis of very regular technical exchanges.

Appendix 3 Architectural options and constraints

The Elstree site will be assumed to be satisfactory for the reasons outlined in 2.7 but, given the degree of commitment now to rebuilding, early consultation with the Regional or District Planning Officers is advisable.

Consideration will be given principally to the production facility since this is the central element and the one most urgently in need of rebuilding. Its scale will be set by the plasma tonnage to be processed and by how many shifts are operated and affected by whether batch or semi-continuous fractionation is used. As Appendix 2 has indicated, an assumption of 2 shifts per day normal operation and batch processing will be made but with the proviso that a final decision on these assumptions should not go by default.

Regarding the size of the production building, it will require a specific architectural brief and exploratory departmental layout drawings to establish approximately the neat size. The preparation of an agreed statement of need together with operational policies for the building is of paramount importance and should include confirmation and description of the method of fractionation. Such a scientific brief should include a functional examination of the sequence of operations and a description of the environmental conditions sought at each stage and whether the environment will be achieved by the building or the equipment to be installed by the Client. Such a document could lead to the preparation of an architectural brief giving a list of rooms or areas together with notional sizes and functional relationships of these rooms and areas. Decisions regarding the environmental conditions would have to be made in collaboration with the Medicines Inspector. (Appendix 4). For the present it is assumed that the whole of the production building would be air-conditioned.

The Architect should be appointed as early as possible to enable him to take part in the preparation of the Schedule of Accommodation and the Room Data Sheets, and allow the Architect to examine the planning problems to establish the minimum areas necessary. To prepare a brief for such a complicated and technical building will take time which should not be underestimated. It will involve the Client, the Architect and the Engineer in many difficult decisions regarding a range of technical and scientific matters from biochemistry to process control. For a budget cost the very least that is required are buildings of a fairly accurate estimated size. The approximate cost could be calculated and say 50% added for the oncosts of the site. Such a system is used in the early stages of, for instance, a District General Hospital, though in the case of a conventional hospital, costs limits provided by the D.H.S.S. are used to obtain the gross estimated sum.

It is important to establish whether the D.H.S.S. Capital Projects Code would have to be followed and if not what cost approval procedures would be applied.

In a scheme of such complexity and size, not only in the design and construction of the building but in the supply and delivery and fitting of the process plant a management control programme covering the whole project from inception to the commissioning is essential. Only in this way will it be possible to consider the maintenance of the service in the existing plant and to examine on a programme basis a planned increase in the raw material which will be required. This might well prove more critical in the project than the construction and commissioning of the new building.

The following are the general functional headings for separate buildings or groups of buildings on the site:

Plasma Fractionation Production

Quality Control, Assay and Research and Development Laboratories

Administration

Service Engineering - Boilerhouse, Ethanol reclamation, Sewage treatment, etc.

and possibly at some future date -

Cell Culture

The site may not give the opportunity to sub-divide the physical buildings in such a manner but there are a number of advantages. Firstly, some buildings will not require as costly a physical solution as others. Secondly, isolation of certain buildings for functional reasons might be desirable. Thirdly, such separation offers the opportunity to phase the groups of building or parts of the buildings, and, finally, there is a potential construction time advantage.

Given a building cost of the total site of 12 to 15 million pounds exclusive of process plant, equipment and fees, the following time scale seems likely given normal departmental policy:

Brief including project objectives, proposed location, revenue estimates and budget cost for Formal Approval	Autumn 1981
Architectural Brief	Summer 1982
Sketch Plan agreed (at this stage a more detailed design cost and revenue cost will be required for Formal Approval)	Autumn 1982
Tenders Issued	Spring 1984
Start on Site	Summer 1984
Completion of Building	Spring 1987
Commissioning and Licensing	Autumn 1988

No account is taken here of the possible time that might be taken in obtaining departmental approvals. Each time-stage is probably the least possible, particularly the construction period, which assumes an average approximate expenditure per month of £450,000 to £500,000. Such an expenditure will require detailed and accurate preparation both by the client and the design team.

This timescale is not appropriate given the need quickly to establish a better environmental standard during processing and the financial advantages which have been shown to accrue by replacing the imports of plasma fractions.

Some saving in time in principle could be achieved by staging construction. However, the production plant is both the most expensive and the most complex element so that its planning and construction will dominate timing.

With a view to reducing costs and the amount of new construction work, and hence the time needed, an examination of the conversion of existing buildings was made. Most of these will be in full use until the completion of the new production facility. Indeed, in some instances such as the quality control sector, extra space will be required prior to re-building to meet more stringent screening requirements. Therefore, while the option of re-using parts of the existing building is well worth examining, it is likely to be for uses such as laboratory and pilot-scale work, final product storage and possibly for production of diagnostic and reference reagents all of which have less rigorous environmental requirements and are not so closely linked to production plant construction schedules.

If a reduction in the interval before construction of a new facility is to be achieved, it appears that a procedure more akin to that adopted by commercial organizations must be adopted. This would entail obtaining tenders from companies specializing in design and contracting for pharmaceutical plants.

Appendix 4 Manufacturing Standards for Plasma Fractionation

Aside from the requirement for larger quantities of plasma fractions, it is the necessity of achieving consistently acceptable manufacturing standards which gives urgency to present plans.

Procuring of plasma must be regarded in the same way as the processing of sterile pharmaceutical products generally. It must acknowledge that because of their sensitivity to heat the various plasma fractions cannot be heat-sterilized in their final containers. Good Manufacturing Practices therefore require the plasma as a raw material to be as free as possible from microbial contamination. The various processing stages, from thawing onwards, must be done under conditions that provide for a progressive reduction of microbial levels. This concept requires processing to be undertaken in environments that provide BS 5295, Class 2, conditions up to the stage of final sterile filtration and thereafter in environments that provide BS 5295, Class 1, conditions.

The analyses of deficiencies at BPL, Elstree, and PFC, Liberton, read in conjunction with British Standards go some way to defining the requirements of a new facility. However, a distinction must be drawn between the alterations required to bring existing plants up to a tolerable level and the standard to be achieved with a new plant which will reach full-scale operation only in the 1990s.

The detailed impact of meeting high environmental standards can only be defined once architectural plans have been drawn up, but it is clear that the cost will be high compared with a simpler process plant. The expense does not derive just from the building finish and the air quality required. The product line must follow a linear path within the plant without back-tracking across or against relatively less clean wastes, personnel movement between these areas must be regulated and changing facilities provided at the interfaces. It must be possible partially to close working areas for cleaning and maintenance and, as indicated in Appendix 2.2, two-stream operation in the precipitation areas would particularly assist this goal.

The incompleteness of process design data for plasma fractionation requires the selection of a more conservative design. In these circumstances the construction of the building must preserve the greatest possible degree of flexibility with respect to technological innovation.

This will best be served by a shell within which a high standard of air-conditioning can be achieved at all points. A layout in which isolated areas of high quality are interspersed with regions which cannot achieve high standards will not allow change except with considerable expenditure and dislocation. Some areas, such as warehousing, do not require air-conditioning and can be grouped accordingly. In principle, Medicines Division would not be opposed to the use of the existing building for warehousing if this proves to be an economic solution. Similarly, Medicines Division will evidently not insist upon laminar flow rooms for processing but rather conventional air filtration with air entering at ceiling level through terminal Hepa filters and exhausted at low level.

Great importance is attached to the quality of protective clothing, the way it is worn and the manner and frequency with which it is replaced. As well as the requirement for changing areas between sections of differing environmental standard, this raises the issue of laundering. The requirement is for specialist laundering of protective clothing which must be kept separate from other laundry and yield a lint-free quality. The difficulty of achieving this in an outside establishment and the high cost of disposable clothing suggests that a facility on the Elstree site should be considered.

The lack of experience of commercial good manufacturing practice and of process engineering among DHSS staff could represent a serious bottleneck in the briefing of contracting engineers and in the necessary discussions with Medicines Division. It makes the role of the plant engineer appointed extremely critical. The appointee will need to be both tireless and strong-willed if a myriad of important details are to be properly thought through. Any miscalculation in this appointment is likely to lead to a plant arbitrarily based on previous ones or left in the hands of contractors who cannot be fully conversant with such a specialized field.

Appendix 5 New fractionation technology

A number of fractionation technologies other than cryo- and ethanol precipitation and limited ion-exchange chromatography are used either in other countries or are in development at Elstree and elsewhere. A full discussion of these would be lengthy and consideration here is limited to those methods which could affect the design of the new plant or are likely to develop in a way which would require thought to be given to their integration into the design during planning or shortly after commissioning.

Other plasma precipitants are used, for example rivanol, ammonium sulphate and polyethylene glycol. However, they do not seem to have outstanding advantages and are not likely to be adopted in the foreseeable future. They could be used in the plant without major changes.

Ion-exchange is already used to purify several components such as Factors VII and IX. The principal suppliers of these chromatographic media, Pharmacia, were invited to present written and verbal evidence to the Working Party and did so. They noted that ion-exchange is in use in a number of countries for albumin purification. Most of these examples involved rather small-scale production. Members were concerned at the time for which plasma remained in the chromatography columns, the cost of chromatographic media, given the potential for fouling by proteins, and particularly by the strategic question posed by production of the material at a single site in Sweden. These fears were not allayed by discussion and it was felt that a gradual incorporation following the present trend was more appropriate than replacement of precipitation technology.

Other chromatographic methods such as gel filtration, immunoadsorption and affinity chromatography are being examined for specific separations, as is the use of solid phase polyelectrolytes, for example, in the preparation of Factor VIIIc. Such methods require no special services or load-bearing floors so that the low temperature environments available for precipitation technology can readily incorporate them.

Perhaps the greatest danger is that, in coping with the pressing problems of the design and construction of a new facility, the development of better technology at Elstree and elsewhere will be given insufficient attention and research will become separated from the main stream of plant planning. To avoid this, some regular method of assessing the consequences of research seems necessary. It should be by a mechanism which avoids discussion being shelved whenever crises to do with the redevelopment programme occur.

Appendix 6 Data Management

Substantial potential exists for the application of computers, microprocessors, etc., in the production of plasma products. Areas which can benefit from this technology include:

process control
quality control
stock control
information handling

The control engineering requirements for plasma fractionation are wide-ranging throughout the whole plant complex. As well as the primary fractionation process control requirement within the plant, there are multiple individual but in some cases highly interactive systems all requiring the same degree of control function. For example, sterilization and pasteurization processes and freeze drying all require control of variables to maximize plant efficiency. The areas of environmental control and energy conservation also have a demanding control requirement. There is the requirement of data acquisition, data reduction and reporting systems to satisfy the requirements of the Medicines Division and effective management of the plant.

With these requirements it becomes essential to consider the whole complex from a process control and data acquisition standpoint in terms of computer technology in its broadest sense.

Process Control

The aim of any control system for fractionation is to reduce operating disturbances which affect overall process performance and the economics of the plant operation. While the fractionation process can be considered simple compared with many chemical and industrial processes, the sequential nature of the process together with the close tolerances required on the relevant operating parameters require an "automatic" process control system to be an integral part of the plant design. This is not to say, of course, that operator involvement in the control system is removed. Indeed, additional measuring and status information must be made available to the operator in order to provide him with the means to monitor the state of the process and, if necessary, to decide on control actions.

There are obviously many ways of fulfilling the control requirements ranging from the installation of a central "all embracing" computer/process

instrumentation interfacing system to multiple local microprocessor installations. A detailed engineering evaluation will be necessary to determine which is the correct system for the complex. Such factors as loading of the microprocessors, requirements for information sharing, display formats, sequencing capability, etc., will all need detailed analysis.

Until the process design, utilities requirements, service engineering facilities have been defined it is impossible to be definitive. It seems reasonable to assume that the desired mode of operation would be from a central control room area but there will exist some need for local "satellite centres". Some of the main system requirements would seem to be as follows:

The system must be:

1) Flexible and easily modified to meet changing process needs and/or process optimization.

2) Easily expansible in terms of hardware and software to meet the requirements of future development.

3) Able to incorporate diagnostic circuitry to detect component failure, alert the operator and so limit the effect on the process.

4) Such that failure of one part does not affect other functionally independent parts of the system.

5) Designed so that total failure does not prevent manual operation of the process.

In addition:

6) Efficient operator/process communication must be provided.

7) Because of the nature of the plant it seems reasonable to assume that certainly during parts of the commissioning period a greater than usual amount of information will be required. Allowance should be made for this. Perhaps trend recorders may be required.

8) In view of the large amounts of process, quality control, environmental, etc., data being acquired, a secure, efficient system of data reduction storage will be required. Sophisticated means to interpret the same may be needed.

9) For economy of installation the system should employ a "data bus"

concept and as much as possible take advantage of multiplexing technology to minimize field wiring.

10) As much as possible the system should be CRT based.

11) Plant safety should be provided by a conventional, separate, stand alone, hard wired emergency shutdown system complete with audible alarm. This should not be connected into the process control system and should not be taken from any transmitted signals.

In applying process control technology two distinct approaches can be distinguished. One where the basic process technology remains unchanged, with manual manipulations being simply automated, and another where the process itself is redesigned to accept better the benefits of control technology.

In plasma fractionation the first approach is only now beginning to be adopted by major manufacturers. Here the large-volume batch technology is retained but with, for example, automatic temperature sensing used to control refrigerant flow and with associated equipment (eg pumps, valves, agitators, centrifuges) being actuated from a remote control panel. Process control of this kind has the advantages of being more reliable and precise than the human operator working in an uncomfortable environment and of allowing the whole plant to be managed from a single station, remote from the relatively open process. Because of the volumes involved, a significant delay exists before any change can be fully sensed and the lag times introduced into a feedback control loop are such that limited improvement in the degree of control can be achieved.

A change from batch to continuous-flow processing allows the in-line sensing of variables such as pH and temperature to provide the basis for feedback control loops. By this means parameters can be held within finer limits and the values can be monitored and recorded for each element of process liquor. With control close to the desired value, the resulting plasma fractions can be more precisely defined. Scottish experience suggests loss of albumin into Fraction IV may be reduced to a point where the development of a waste recovery system is no longer necessary.

Most of the plasma received for fractionation can be placed in one of three categories:

A Fresh frozen, in 5L packs or single donations, primarily for preparation of Factor VIII and Factor IX complex;

B Specific antibody, single donations or larger packs, primarily for preparation of specific immunoglobulins;

C Plasma separated from time-expired blood and supernatant from cryoprecipitate (prepared at RTCs).

Normal immunoglobulin may be prepared from A, using the supernatants and from C. These may be combined. Albumin or PPF may be prepared from A, B and C, that is from all source material. The kind of flow pattern which is associated with these various separations operating on a large scale will call for detailed programming with suitable interlocks so that processing of material of the three types is maintained without unduly long holding periods at intermediate stages. A computer programme to enable the separate fractions for various products to be linked in time and volume according to priorities will simplify the preparation of weekly or monthly work schedules.

Quality Control

To achieve complete quality assurance it is essential that defined limits be attained in all aspects of the production operation. Reagents, components, in-process materials and operations, the environment, packaging materials and the final product must all meet specifications defined in physical, chemical, biochemical or microbiological terms. In addition, the full history of individual product lots (including input plasma, materials and components used, processing details, etc.) must be retained for records purposes and be easily available. Details of all these items and procedures must also be fully documented and this must be continually revised and updated. The data handling associated with these tasks is considerable and modern systems will be essential for the full and proper use of this information.

Information Handling

The preparation of clinical products from human plasma encompasses a wide range of scientific and technical disciplines and in a modern Centre an awareness of developments in all of these areas is important. The retrieval and storage of this information must be undertaken and a substantial volume of in-house documentation must also be controlled. Modern information-handling systems have the capacity and flexibility to allow easy access to all of this information but the introduction and development of the computing and control systems noted above requires the full-time employment of specialist staff at an established professional level.

Appendix 7 Plasma Supply

The main factor determining plasma supply is the requirements for Factor VIII as freeze-dried intermediate concentrate. If the capacity set for the new production facility is 90 m units pa, a supply of 450,000 litres of frozen fresh plasma would be needed, allowing for a projected final product yield of 220 units per litre. A realistic initial production capacity will have to be assigned and a projected expansion profile established. At present this is clearly hampered by lack of information on dates, but, the shorter the interim period to commissioning, the greater is the requirement for readjustment within the routine blood donor programme of NBTS. For example, within three years it is likely that the only areas for improvement would be a greater proportion of the existing total blood donations set aside for fractionation and the development of NBTS policy on plasmapheresis and some other related matters. Within five years it would be realistic to expect FFP supply from 60% of the total donor programme and a significant input of plasma from plasmapheresis. Coordination of timing will be critical between plasma supplies and fractionation development.

Assuming a capacity of 200,000 litres FFP at the time of commissioning new plant, Factor VIII yield would be 44 M units, which is still well below current rate of use ($\simeq$ 60 M units); thus a need to progress rapidly from the commissioning production capacity would be essential if the financial returns on the project investment were to be realized.

A decision by NBTS directors to phase out the 5 ℓ pooling of plasma at regional centres has resulted in a project to develop a single pack which accommodates a collection of desirable features and aims at both regional centres and BPL. The first regional trial of 6000 single plasma packs (SPP) is taking place.

The SPP is designed mainly to cope with special requirements of fresh frozen plasma (FFP) unit collection in larger numbers from the normal blood donor programme and to preserve that plasma in a state of uniform high quality throughout NBTS for fractionation, primarily of factor VIII at optimum yield. It is anticipated that benefits of high quality FFP feedstock will also be reflected in quality of finished product, both in coagulation factors and Cohn fractionated proteins.

There is positive identification of each donation until immediately before fractionation. This control requirement may become essential

for regulatory purposes. The SPP will incorporate the bar-code system of pack and donor identification into control procedures at BPL and into pre- and post-quarantine cold storage. It is designed for automated opening at BPL, allowing rapid accumulation of FFP in collecting systems with a high degree of environmental protection of both feedstock and operator. The pack-opening procedure will permit decoupling of this procedure from the time-schedule of the fractionation programme, i.e. the initial feedstock handling can be designed and organized as a separate entity with its own "holding-capacity". Commercial tear-down equipment specific to SPP handling is undergoing trials and in addition the Edinburgh Protein Fractionation Centre is examining the use of an automatic dipping machine in use in the food industry which could handle 500 kg plasma/hr and facilitate stripping of the plastic bag by warming in aseptic solution.

At present, provision for handling the SPP at BPL will not exclude use of the 5 ℓ pack for time-expired plasma, although this form of feedstock should reduce sharply if 60% or more of the whole-blood donated is used for FFP. Equally, during its introduction, FFP in both SPP and 5 ℓ packs will need to be accommodated. By the time a new laboratory is built, the supply problem for FFP should have improved to a level which excludes the bulk of time-expired plasma currently received.

New plasmapheresis equipment permits the removal of 500 ml plasma in approximately 30 minutes and is likely to be more acceptable to donors than conventional plasmapheresis in a standard "closed" quadruple pack. Unless considerable red cell wastage is to occur, the large deficit in plasma requirement for fractionation must be met by plasmapheresis in some form. Plasma obtained by plasmapheresis can be frozen rapidly after collection and has the potential for high quality in terms of its labile factors.

A new system of plasma separation by membrane-pheresis will soon be available. The new method depends on ultrafiltration of plasma across a membrane; thus the system is free of centrifugation and the plasma is free of cells and supported in the anticoagulant of choice. In both plasmapheresis systems, the plasma container may need modification to permit easy tear-down at BPL.

A change from 5 ℓ pooled plasma to the SPP or plasmapheresis

systems will have a primary impact on technology in the area of thawing and cryoprecipitate harvesting. It is assumed here that the automation programme for opening SPPs or other single plasma units will be separated from the immediate area of cryoprecipitate production.

For pack-opening of SPPs, the prototype machine requires an area where the SPP is released from its outer wrap and fed to a room housing the tear-down machine. Environmental requirements for the tear-down room are Class II with positive pressure to the ante-room. Ideal design will allow for extruded plasma blocks to transit through a partition separating the tear-down room from a small Class I refrigerated area concerned with "bagging-up" and sealing fixed weights of FFP blocks. Access from this area into a cold storage holding facility pending fractionation will complete the functional content.

The growth in regional supply of FFP to BPL will require reorganization of existing systems for dispatch or storage. Careful costing is needed to determine whether, if the task is retained at regions as at present, growth in cold storage capacity or more frequent dispatch, with consequent increases in transport needs, will prove more economic. Alternatively, centralization of transportation and storage at Elstree may effect savings in regions and allow for a more rationalized and controlled programme giving better protection to product.

Appendix 8 Developments in genetic manipulation

Recent scientific advances in the manipulation of genetic material have led to technological developments introducing the possibility of large-scale production of proteins for therapeutic use from bacteria or from animal cells cultured in vitro. The extension of this work to the production of human proteins is receiving a great deal of attention and there have been two major lines of work towards this end. One has drawn on recombinant DNA techniques to construct bacteria capable of producing "human" proteins. The alternative has been to change the characteristics of animal, including human, cells to make them more amenable to large-scale cultivation in vitro. An example of this approach is the production of monoclonal antibodies by cell hybrids. Related to this have been advances in the technology of in vitro animal cell culture that make it possible to consider using normal cells as the basis of a large-scale protein production system.

Clinical trials of human insulin and urokinase prepared by such technologies are imminent. Workers of the Upjohn Company have succeeded in inducing the production and secretion of chicken ovalbumin in E. coli using recombinant DNA technology. There is preliminary evidence that what are currently waste materials from ethanol fractionation of plasma may be used in the culture of mammalian cells.

It is necessary to decide whether the principal plasma proteins could be generally available at a competitive cost and of proven safety in a time-scale which bears upon the development of BPL. If so it is important to decide whether the technology arising from genetic manipulation is one which BPL would logically become engaged in.

There are several potential advantages to in vitro production of proteins for therapeutic use. The cells used in large-scale culture systems will be subject to intense selection at an early stage and will be a clone arising from a single cell that has the most desirable combination of characteristics. These characteristics will include the plentiful production of the required protein, preferably secreted into the culture medium with the least possible production of other proteins and macromolecules. Consequently recovery of the protein product will be from a much less complex mixture than, for instance, the plasma from which the blood products currently available are prepared. This could mean that very much more simple recovery systems will be required leading to a substantial saving in processing costs.

Each of the systems that have been proposed as the basis of an in vitro protein production system have particular disadvangages that may restrict the use of their products. Apart from albumin virtually all of the protein species present in plasma are glycoproteins or lipoproteins. The carbohydrate or lipid in these macromolecules seems to be important in vivo particularly in respect to modulation of the antigenicity and pharmacokinetics of the active protein. It is doubtful that bacteria will be able to reproduce these features faithfully and so it will become necessary to introduce in vitro modification into the processing of the product. Further, bacterial proteins are intensely antigenic in mammals and cell débris is pyrogenic. Such material will inevitably be present in the crude product from bacterial systems and as even trace levels of this contamination repeatedly administered over a long period, as in the control of haemophilia, may be dangerous, these products will have to approach absolute purity. As greater purity is sought with large-volume processes costs rise exponentially and consequently the processing cost may offset the initial production cost advantage of bacterial systems.

Animal cell systems have the advantage that the products are likely to be correctly modified and to be secreted directly into the culture medium with a significant reduction overall in antigenicity and pyrogenicity. However, cell lines that will grow continuously in culture without ageing are either transformed or are a hybrid of a transformed and normal cell. Thus there is concern that their products may contain a transforming agent capable of producing tumours in recipients. The transforming agent may be sections of DNA or maybe other large or small molecules. Production from normal cells could avoid this danger but, although rapid progress is being made, the conditions for their cultivation are more complex than for either bacterial or transformed cells and are at present less well defined.

The homogeneity and purity of proteins synthesized in vitro will make them particularly potent therapeutic materials, both in regard to beneficial and detrimental effects on the patient. The beneficial potency is the basis of the clinical value of these products; however, untoward side-effects arising from that same potency may considerably complicate their use. Genetic polymorphism of plasma proteins is now well established with, for example, more than 20 variants of human albumin identified to date. Thus, as with any other tissue, it is possible to characterize any individual according

to their "plasma protein-type". Administration over a prolonged period of homogenous proteins that do not match the recipients plasma protein-type could induce the synthesis of neutralising antibodies which would result in very severe complications. Avoiding or minimizing this hazard could require production from several cell strains, each producing material of a different plasma protein-type. This difficulty is reduced with the plasma protein preparations currently available because they are prepared from mixed pools of donated plasma and thus contain the whole spectrum of plasma protein-types, with none being unduly emphasized. The use of genetically engineered products will require information as to the characteristics of the patient and of the protein to ensure their compatibility and also constant monitoring of the patient's condition for evidence of side-effects. Very close liaison will be necessary between the clinical users and the producers of these materials, with support from comprehensive analytical laboratories to ensure the close matching of patient and medication and the rapid production of an appropriate variant when one is not already available.

The successful development during the last 30 years of a range of therapeutic products prepared by the separation of whole blood into its components has involved contributions from several disciplines within the Blood Transfusion Services. The development of the modern processes for the production of plasma protein concentrates has involved the extensive application of process and biochemical engineering in the handling of large volumes of fluid while preserving the biological activity of the components. The laboratory staff are well acquainted with the difficulties in the reliable measurement of labile biological activity and with the techniques of typing tissue antigens, which underlie successful blood transfusion. Finally, the clinical staff are familiar with the uses and hazards associated with these products and with the management of patients receiving them. Given that such developments are likely to become significant only by the late '80s it would be possible to embrace them within future plant and personnel planning. Incorporation of tissue culture and especially of microbial culture onto the Elstree site would require careful planning and would certainly increase effluent disposal problems, but preliminary consideration does not indicate any absolute objections.

In summary, genetic engineering is proceeding towards application at

a rate that requires it to be seriously considered. Given prompt action on the redevelopment of conventional fractionation facilities, the return on investment will have been achieved before genetically engineered products can have a major impact. Nevertheless, failure to take account of them may lead to a crisis shortly after the new plant has commenced operation.