III.—New Developments in Large-scale Plasma Fractionation. By J. G. Watt, J. K. Smith, W. Grant and C. Turnbull, Scottish National Blood Transfusion Association, Protein Fractionation Centre, Royal Infirmary, Edinburgh. (With 1 plate and 14 text-figures)

'Selective transfusion of appropriate blood components is preferable to the routine use of whole blood.' (American Association of Blood Banks. *Physicians' Handbook of Blood Component Therapy*, 1969).
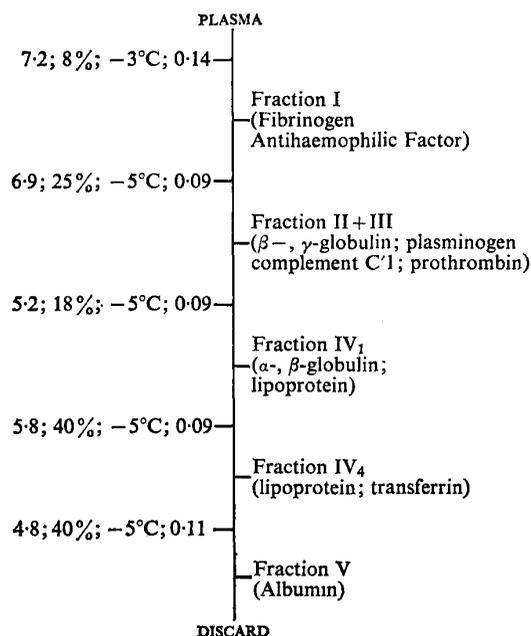
1. INTRODUCTION

The rational use of donor blood in the environment of modern blood transfusion revolves around a series of developments in the field of component therapy. The fractionation of plasma, i.e. the separation of plasma into a series of subdivisions each containing one or other of the constituent proteins in varying degrees of purity and concentration, was one of the first of these developments to become established.

Early fractionation of plasma was applied in various ways to the purification of animal antisera; initially to antipneumococcal serum for therapeutic use. These sera frequently provoked reactions in patients until Sabin showed that absorption with Fullers earth could abolish these side-effects by removing complexed molecules.
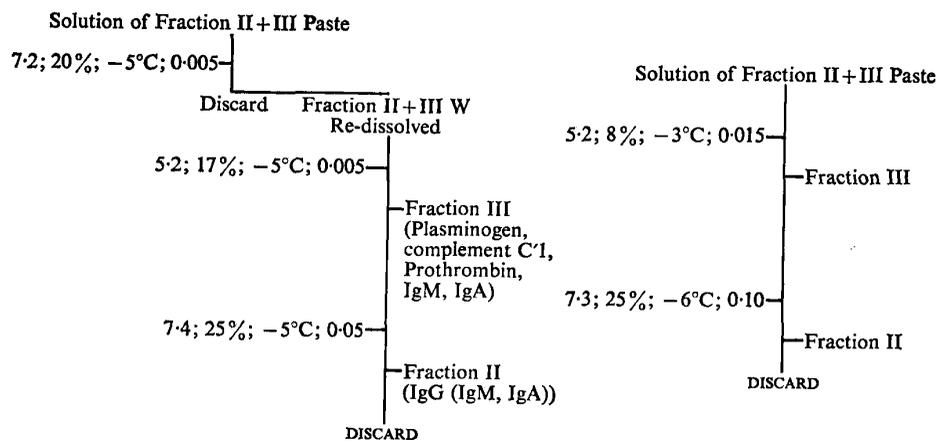
Tiselius, in 1938, showed by his newly invented moving boundary electrophoresis that antibodies were associated with the fraction which he called gamma-globulin and about the same time Edwin Cohn, in Boston, was developing techniques of separating plasma proteins to facilitate his studies of their chemical and biological properties. His expertise was harnessed to clinical application when it was directed toward the discovery of a stable volume expander to replace gum acacia, hitherto used by the US Navy. The method of organic solvent precipitation (text-fig. 1) provided the needed requirement in the shape of human albumin. The Cohn method had several advantages:

1. The use of a cheap precipitant (ethyl alcohol) allowed the process to operate at low temperatures and minimised the risk of bacterial growth.
2. Lyophilisation could be used to remove the final traces of alcohol from the precipitated protein.
3. Manipulation of five different variables, allowed maximal opportunity for separating one protein from another on the basis of solubility differences:

    (i) Hydrogen ion concentration.
    (ii) Alcohol concentration.
    (iii) Protein concentration.
    (iv) Temperature.
    (v) Conductivity.

The Cohn procedure underwent many modifications to provide a range of fractions. Of these the fractions I, II and V were quickly shown of clinical value in their provision of fibrinogen and antihaemophilic factor; IgG antibodies and albumin. As the method

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PLASMA

7·2; 8%; −3°C; 0·14—

Fraction I
—(Fibrinogen
Antihaemophilic Factor)

6·9; 25%; −5°C; 0·09—

Fraction II + III
—(β−, γ-globulin; plasminogen
complement C′1; prothrombin)

5·2; 18%; −5°C; 0·09—

Fraction IV₁
—(α-, β-globulin;
lipoprotein)

5·8; 40%; −5°C; 0·09—

Fraction IV₄
(lipoprotein; transferrin)

4·8; 40%; −5°C; 0·11 —

Fraction V
(Albumin)

DISCARD

TEXT-FIG. 1—Scheme of Method 6 alcohol precipitation system (Cohn *et al.* 1946). Values of pH, alcohol concentration, temperature and ionic strength are shown on left of main fractionation sequence. This method is used almost universally in North America and in Europe to some extent.
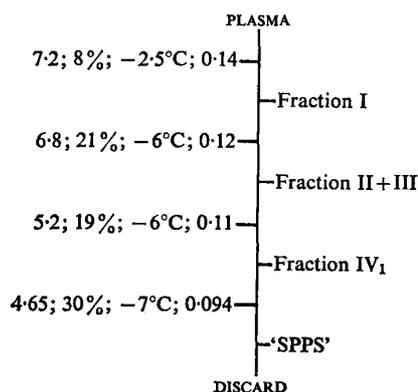
Solution of Fraction II + III Paste

7·2; 20%; −5°C; 0·005—

Discard   Fraction II + III W
Re-dissolved

Solution of Fraction II + III Paste

5·2; 17%; −5°C; 0·005—

5·2; 8%; −3°C; 0·015—

—Fraction III

—Fraction III
(Plasminogen,
complement C′1,
Prothrombin,
IgM, IgA)

7·3; 25%; −6°C; 0·10—

7·4; 25%; −5°C; 0·05—

—Fraction II

—Fraction II
(IgG (IgM, IgA))

DISCARD

DISCARD

TEXT-FIG. 2.—Schemes for sub-fractionation of fraction II + III to provide fraction II which is composed mainly of IgG with traces of other immunoglobulins. These could be applied to sub-fractionation of the schemes shown in text-figs. 1 and 3. (Oncley *et al.* 1949; Krijnen *et al.* 1970). Values for pH, alcohol concentration, temperature and ionic strength are shown on left of main fractionation sequence.

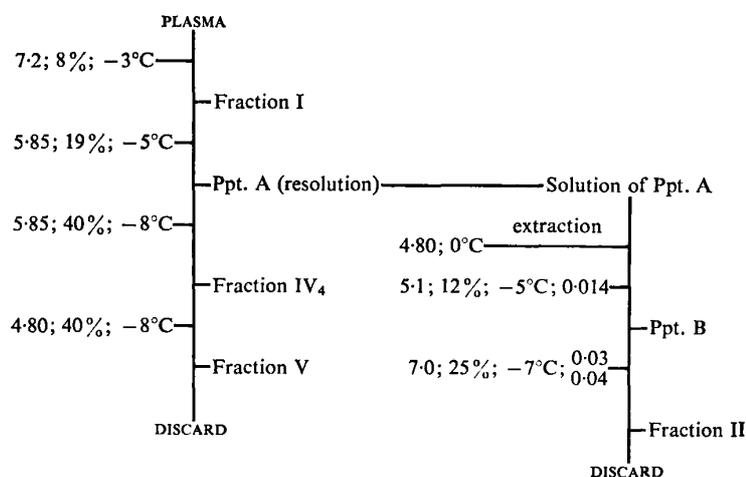New Developments in Large-scale Plasma Fractionation   s 17

came to be widely used and commercially exploited in the United States during the Second World War it underwent a series of further changes and modifications until, at the present time, a range of different forms of alcohol precipitation are in existence. Text-figures 2, 3 and 4 illustrate some of these modifications.

Other techniques have been developed (text-fig. 5) but none have been so widely adopted as the Cohn system and its variants.

The Cohn system was adopted in Edinburgh in 1951 on a modest scale as part of the activities of the Regional Transfusion Centre and, developed steadily in terms of scale and range of products (text-figs. 6–12) until it became recognised that international acceptance of a plasma volume expander of plasma origin, but safeguarded

PLASMA

7·2; 8%; −2·5°C; 0·14—

—Fraction I

6·8; 21%; −6°C; 0·12—

—Fraction II+III

5·2; 19%; −6°C; 0·11—

—Fraction IV$_1$

4·65; 30%; −7°C; 0·094—

—'SPPS'

DISCARD

Text-fig. 3.—Schemes for alcohol fractionation to product 'plasmanate' (SPPS) (Hink et al. 1957), forerunner of Purified Protein Solution (PPS), whilst allowing access to other required proteins. Values of pH, alcohol concentration, temperature and ionic strength are shown on left of main fractionation sequence.
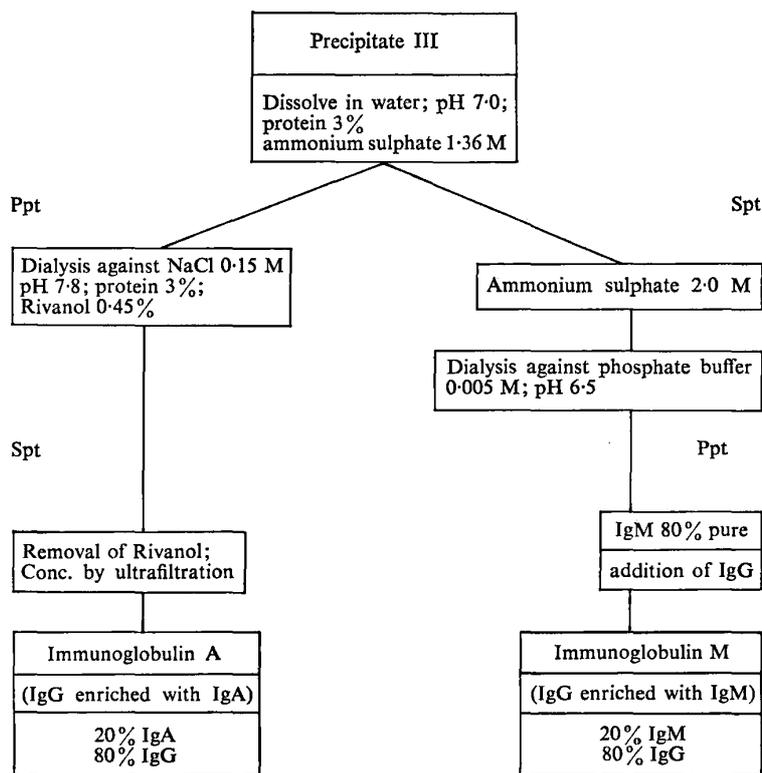
PLASMA

7·2; 8%; −3°C—

—Fraction I

5·85; 19%; −5°C—

—Ppt. A (resolution)————————Solution of Ppt. A

5·85; 40%; −8°C—   extraction
                          4·80; 0°C————————

—Fraction IV$_4$ 5·1; 12%; −5°C; 0·014—

4·80; 40%; −8°C—   —Ppt. B

—Fraction V   7·0; 25%; −7°C; $\frac{0·03}{0·04}$—

DISCARD   —Fraction II

DISCARD

Text-fig. 4.—Scheme devised (Kistler and Nitschmann 1962) as modification of Cohn technology.

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against the risk of transmission of serum hepatitis, would necessitate expansion of the processing facility. At the same time it was becoming clear, from the work of Blomback (1958), Blatrix and Soulier (1959), Pool and Robinson (1959), Wagner (1962) and Johnson, Newman, Howell and Puszkin (1966), among others, that the range, quantity and purity of concentrates of coagulation factors becoming available for clinical application would demand a technology which, in part at least, would necessitate a departure from systems entirely dependent on alcohol precipitation.

Although, during the last two decades, there had been some interest and manufacture of immunoglobulin fractions from plasma known to contain certain antibodies, this divergence had not been widely exploited in the Edinburgh laboratory. The introduction of prevention of rhesus immunisation in susceptible women by the use of specific immunoglobulin (Finn 1960; Clark 1969) and the burgeoning need for
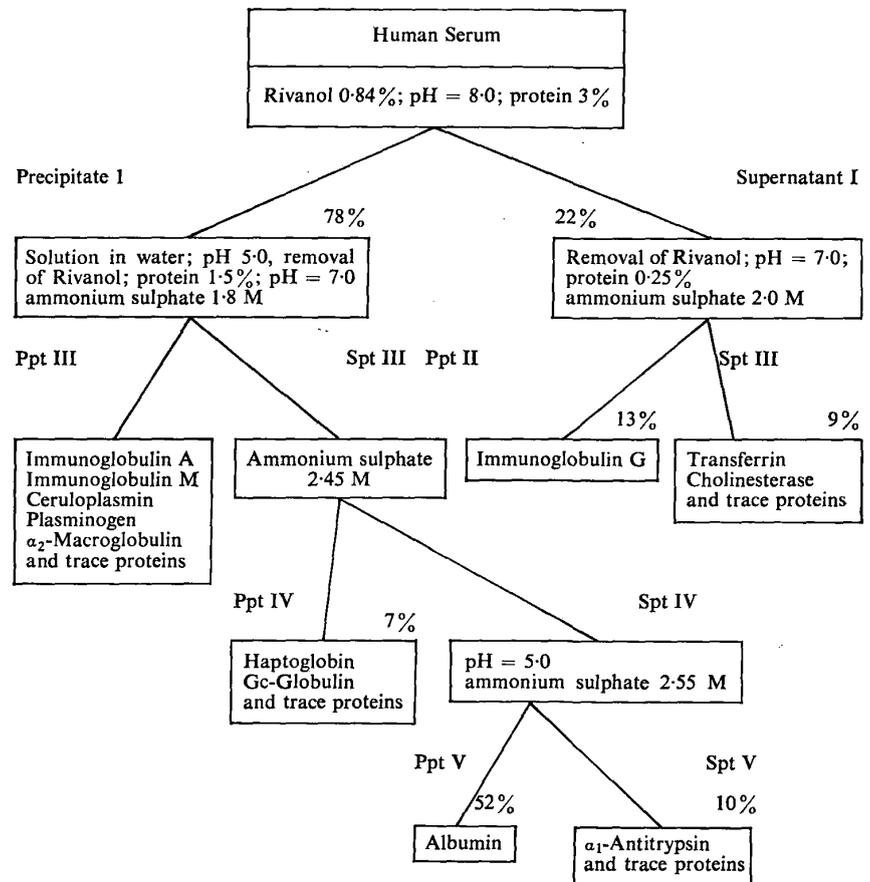
Text-fig. 5a.—Combination of rivanol and ammonium sulphate precipitation for production of immunoglobulin (IgG) and albumin (Schwick 1970).

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                    ┌─────────────────────────┐
                    │     Precipitate III     │
                    ├─────────────────────────┤
                    │ Dissolve in water; pH 7·0; │
                    │ protein 3%              │
                    │ ammonium sulphate 1·36 M │
                    └─────────────────────────┘
        Ppt                                        Spt

┌───────────────────────────┐          ┌───────────────────────────┐
│ Dialysis against NaCl 0·15 M │        │ Ammonium  sulphate  2·0  M │
│ pH 7·8; protein 3%;        │          └───────────────────────────┘
│ Rivanol 0·45%              │          ┌───────────────────────────┐
└───────────────────────────┘          │ Dialysis against phosphate buffer │
                                        │ 0·005 M; pH 6·5           │
        Spt                             └───────────────────────────┘
                                                              Ppt

                                        ┌───────────────────────────┐
                                        │      IgM 80% pure          │
                                        ├───────────────────────────┤
┌───────────────────────────┐          │    addition of IgG         │
│ Removal of Rivanol;        │          └───────────────────────────┘
│ Conc. by ultrafiltration   │
└───────────────────────────┘

┌───────────────────────────┐          ┌───────────────────────────┐
│    Immunoglobulin A        │          │    Immunoglobulin M        │
├───────────────────────────┤          ├───────────────────────────┤
│ (IgG enriched with IgA)    │          │ (IgG enriched with IgM)    │
├───────────────────────────┤          ├───────────────────────────┤
│       20% IgA              │          │       20% IgM              │
│       80% IgG              │          │       80% IgG              │
└───────────────────────────┘          └───────────────────────────┘
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TEXT-FIG. 5b—Ammonium sulphate and rivanol precipitation system for preparation of macro-globulin (IgM and IgA) enriched immunoglobulins (Schwick 1970).


Normal Immunoglobulin

300 mg

750 mg
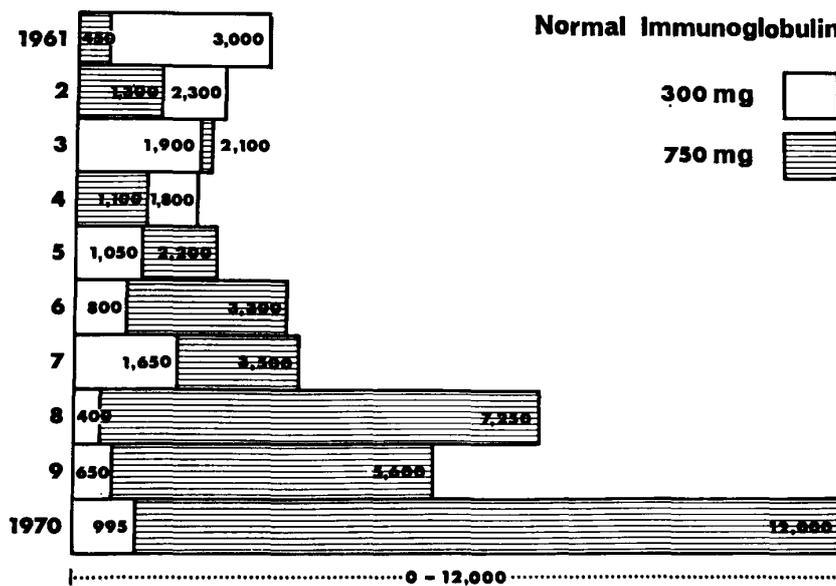
TEXT-FIG. 6.—Production of plasma fractions in Scotland. Production of immunoglobulin (fraction II) 1961–1970.

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immunoglobulins with specific activity against tetanus toxin and vaccinia virus recreated the need for expansion of immunoglobulin production.

These factors all played their part in the adoption of a development programme which was initiated in 1967 and which will culminate in the commissioning of new processing facilities which are now under construction on a site near Liberton Hospital on the south side of Edinburgh. In the meantime, the development programme has allowed plasma processing to increase in range and volume at an even greater speed so that the situation exists whereby some fractions (normal immunoglobulin, anti-D immunoglobulin and fibrinogen) are being produced in quantity to meet the full needs of the Scottish community and other fractions are available in increasing amount.
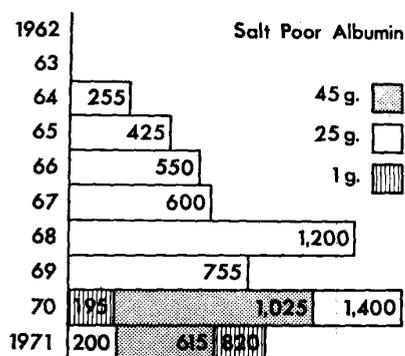
TEXT-FIG. 7.—Production of plasma fractions in Scotland. Production of salt-poor albumin (fraction V) from introduction in 1964–1971.
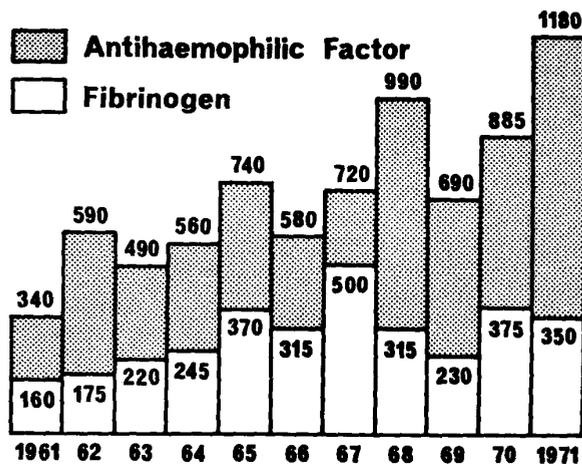
TEXT-FIG. 8.—Production of plasma fractions in Scotland. Production of fibrinogen and antihaemophilic factor (fraction I) from 1961 to 1971.
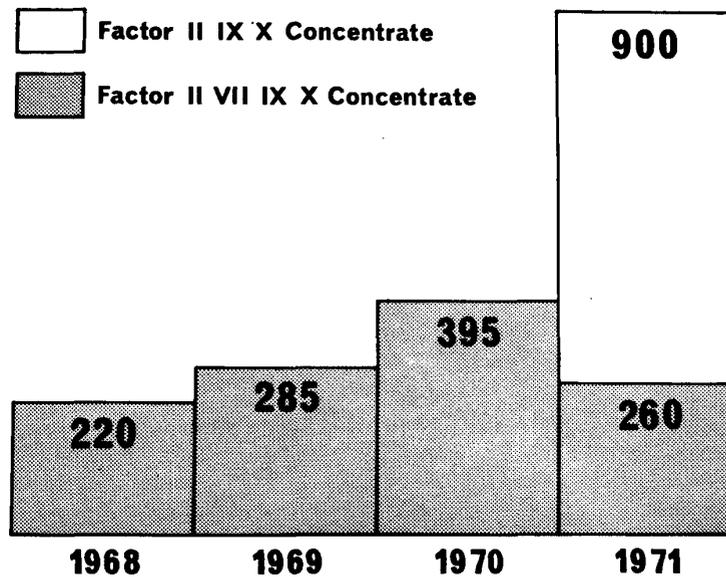
TEXT-FIG. 9.—Production of Plasma Fractions in Scotland. Production of coagulation factor concentrates from introduction in 1968–1971 when full introduction of the concentrate derived from ACD plasma became apparent.
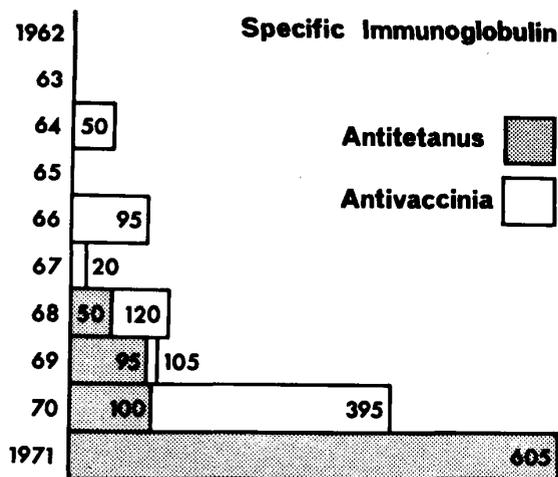
TEXT-FIG. 10.—Production of plasma fractions in Scotland. Production of anti-vaccinia and anti-tetanus immunoglobulin. Erratic character of production arises from the building of plasma pools which were processed as soon as adequate volumes had accrued.

2. Developments of Fractionation Systems

This development has allowed opportunity to re-examine some aspects of accepted methodology and to introduce new techniques:

1. Organic solvent precipitation is being removed from the accepted static batch technique (text-fig. 13) and organised as a continuous process which can be, to a large extent, placed under automatic control using a process control computer (Watt 1970a, 1971). This departure will allow achievement of laboratory standards of precision on industrial scale operation and the introduction of a sixth variable, time, to the Cohn philosophy. Of course, time has always been an existent variable but has not been universally recognised as such, nor have its effects been quantified. The essential difference between continuous and batch fractionation methodology is the realisation that, provided all variables are fixed at correct levels, the precipitation of plasma protein is a near-instantaneous phenomenon (Pl. I).
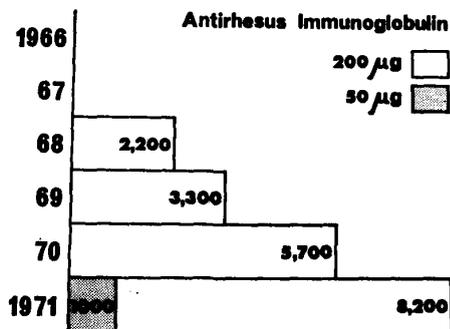
TEXT-FIG. 11.—Production of plasma fractions in Scotland. Production of anti-Rhesus immuno-globulin since introduction in 1967–1971 when processing reached full scale for this product at present need.
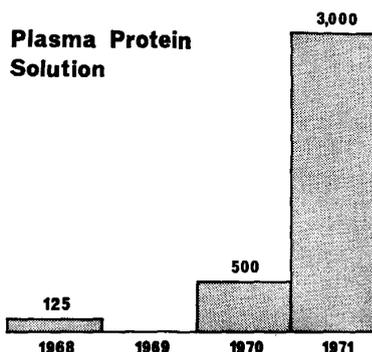
TEXT-FIG. 12.—Production of plasma fractions in Scotland. Introductory production of plasma protein solution. Production in 1968 was exploratory and issue effectively commenced at beginning of 1971.

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WATT et al.   PLATE 1
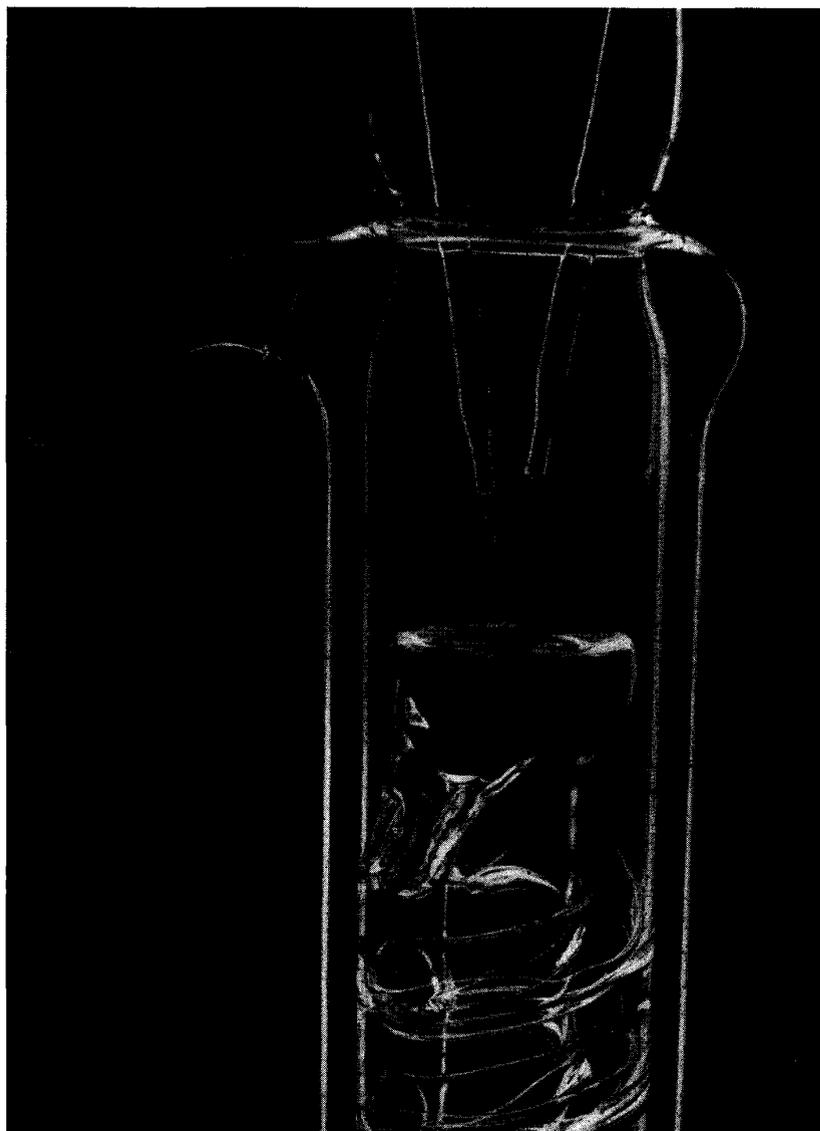
Mixing cell of continuous flow system showing the mixing of plasma and alcohol streams against
the cooled glass surface. The whole process of temperature adjustment and precipitation is completed
before the mixed liquid leaves the cell 6 seconds later.
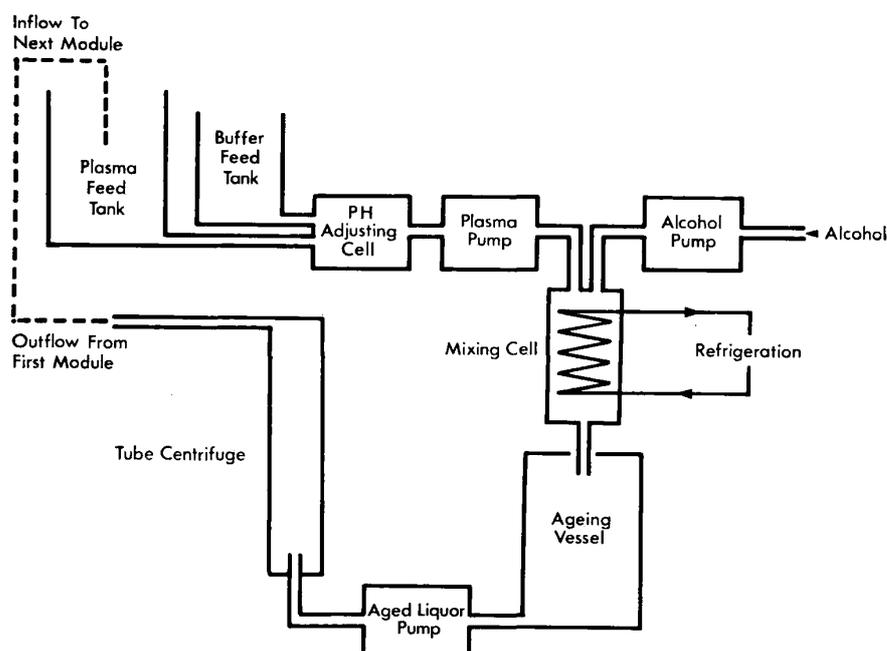
TEXT-FIG. 13.—Sequential arrangement of continuous flow alcohol precipitation system. Monitoring and control circuitry are not shown. Diagram represents one sequence module; five modules are required to process plasma to albumin by Cohn Method 6.

2. The removal of traces of ethanol from albumin preparations using lyophilisation is a slow process accompanied by severe capital and operating costs. The product is subjected to considerable heat, especially during the later stages of the process and a varying degree of damage, in the form of polymerisation, can occur. Vacuum distillation is a process (text-fig. 14) where the protein solution spends a few seconds in contact with a heated surface in vacuity so that heat damage can be minimised (Smith, Watt, Watson and Mastenbroek 1972). Heat damage (polymerisation) is dependent on degree and length of exposure. Ethanol traces in this and other fractions, e.g. immunoglobulin, can be removed by molecular sieve chromatography; the possibilities in this area are being explored. None the less, the removal of the bulk drying problem of albumin allows concentration of lyophilisation effort on the other fractions and, by freeing equipment, allows adoption of less rigorous operating conditions.

3. The final stage of preparation of clinically useful products are concerned with quality control, sterilisation, dose-measurement and dispensation. The advent of membrane filters and the less well-known resin-bonded asbestos and glass-fibre sheets has had profound effects on sterile filtration of plasma fractions. These have allowed the construction of mixed-media filtration programmes which permit the coarser stages of filtration to be done on large-capacity conventional asbestos pads with subsequent final filtration through the more delicate media, often in stages of increasing

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exactitude, to ensure removal of particles shed by the primary filter systems. Many labile factors, e.g. fibrinogen and antihaemophilic globulin, cannot easily be filtered through asbestos pads but may be successfully processed by the more inert media. Thus, items which had to be processed under closed systems of dubious aseptic character can now be safeguarded by final sterile filtration.

The changing character of quality control assessment has posed many problems for the fractionation development programme. The biological activity of coagulation factors has been assessed classically by methods adapted from clinical laboratory techniques but such methods are barely adequate for production quality control on concentrated fractions; they do not provide recovery assays with any reason-
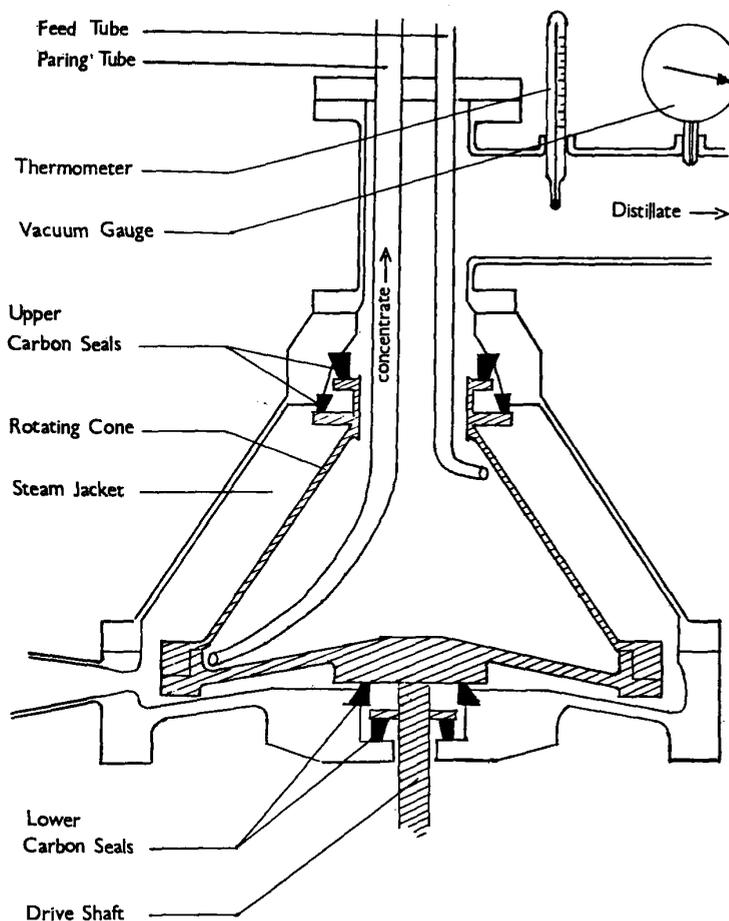
TEXT-FIG. 14.—Cross-section of effective portion of vacuum distillation equipment showing the feed tube which deposits the alcohol-bearing liquid at the apex of a spinning, heated cone and the paring tube which scoops up the concentrated residue at the base of the cone. Liquid in process forms a thin layer on the inner wall of the cone.

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able degree of accuracy. It is only after many recovery measurements on many different preparation batches that it becomes possible to define the acceptance limits of a particular technology. The best assay for coagulation factor concentrates remains, regrettably, the clinical performance of a particular batch of the product concerned; ideally it should be possible to provide a real assessment of the clinical response before the product is used. Retrospective assessment of the clinical response is difficult to organise and operate in practice, however well disposed the individual user may be it is frequently not possible to collect the information required. More frequently the exigencies of treatment are such that interpretation of data is clouded by other events. The effect of administration of concentrate to a haemophiliac with active haemorrhage will be, understandably, less than when haemorrhage is very slight or absent. The administration of other agents such as whole blood may make the assessment of efficacy even more meaningless. However, the lack of such data inhibits progress toward more effective and more available products.

Where immunoglobulin is prepared from non-selective plasma the quality control situation is purely chemical. Provided the final product is sterile and free from pyrogen the producer must only ensure that a given weight of protein is present in the final container. Adequate methods, well tried and accepted, exist for checking the effectiveness of achieving this. The preparation of specific immunoglobulin demands effective bioassay systems and these are frequently subject to error, are cumbersome or require special skills not available except in a few laboratories. There is little doubt that the need to prepare specific immunoglobulin will have to increase both in quantity and variety. New assay systems may be one of the major hurdles faced in such development. Already problems have arisen in preparation of anti-herpes immunoglobulin where the only assay available was rendered useless because of the anti-complementary character of the product.

3. PLASMA FOR PROCESSING

The fractionation of plasma could be said to start at the time the donation of blood is collected from the donor. The time required for the actual donation, the type of anti coagulant into which it is collected, the method of storage, rate of cooling, agitation of the cooled blood, time of separation of plasma from cellular elements, the speed and period of centrifugation, storage of the plasma, rate of freezing and rate of thawing are all factors bearing on the quantity and quality of the products which can be recovered from plasma. In practice the quality of plasma for processing is the result of compromise between the needs of blood transfusion and the ideals of fractionation. If, as happens in one hospital in the United States (Sgouris 1972), all but 2 per cent. of collected blood were administered as packed cells it would be feasible to provide abundant plasma for the preparation of antihaemophilic globulin (AHG). The preparation of this fraction is a good example of the kind of logistic alternatives which are possible and the effects these can have on the supply of the fraction itself.

The pattern of production of antihaemophilic factor in the Edinburgh Centre (text-fig. 8) shows a gradual increase overlaid by considerable fluctuation dictated by availability of suitable plasma whilst the clinical requirement remains unsaturated. By comparison, text-fig. 11, showing the steady production of anti-D immunoglobulin is a demonstration of a planned policy of sufficiency in which the availability of

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suitable plasma and the demands of increasing clinical requirement were equally controlled until, by late 1971, the full clinical need has been satisfied.

The original AHG concentrate was Cohn fraction I made from plasma separated from blood within 6 hr of collection. The plasma, to obtain good yields of AHG has to be platelet-free. A programme to provide platelet concentrates will not, at the same time, provide good starting plasma for AHG preparation because it is almost impossible to make platelet-free plasma from platelet-rich plasma and the time-scale of preparation of platelets is usually such that marked loss of activity would occur in any case.

The advent of cryoprecipitation (Pool and Robinson 1959) allowed transfusion laboratories to produce an AHG concentrate which, in terms of recovery of coagulant activity is inferior to the Cohn fraction but, because it can be prepared with little capital investment and because the areas of production and use were closely associated this deficiency could be repaired by processing extra plasma. There can be no doubt that, on a world-wide scale, cryoprecipitation has allowed treatment and prophylaxis of haemophilia on a scale which was previously impossible.

The social convenience of cryoprecipitate for the haemophiliac has, undoubtedly, been a major factor in its acceptance but in this regard the most successful variant is the lyophilised version which can rarely be prepared in a transfusion centre. However the real factor of concern must be the translation of factor VIII activity in plasma into factor VIII activity in the patient through the medium of a concentrate. The activity in plasma may vary between 50 units and 200 units (where a unit is defined as the activity in 1 ml of 'normal' plasma) and the coefficient of variation in the assay system used is of the order of ±45 per cent. (Pool 1967). These variables must be discounted in calculation of the various alternatives although, ultimately, the level of factor VIII in the starting plasma cannot be ignored.

The major problems in preparing fresh plasma are the availability of adequate centrifugation capacity and the amount of blood which can be separated within the 4-h-post-collection period. If the 'period of grace' is expanded to 18 hr this particular restriction is largely removed since it becomes more a matter of providing labour over longer working periods. In activity terms the provision of 10 litres of plasma at 0·8 units/ml is the same as 16 litres at 0·5 units/ml. However, neither of the processing systems mentioned are capable of making good, clinically acceptable, concentrates from low activity plasma; thus new technology must be adopted. For this the method of Johnson, Karpatkin and Newman (1969) seems ideally suited and modifications of this system seem even better (Johnson 1971; Bidwell 1971, personal communications). Preliminary experiences with this product show promising results although, as yet, we lack data on clinical application.

Plasma, stored as whole blood longer than 24 hr, shows a steady loss of AHG activity but none the less it is interesting to speculate that it may be worth, in routine processing, making apparently poor recoveries if the final product can be concentrated 1000 times or more.

Similarly, in consideration of concentrates containing the coagulation factor IX, one may be forced to consider alternatives. The calcium phosphate adsorption technique (Didishiem, Loeb, Blatrix and Soulier 1959) provides a very successful product (PPSB) but requires the collection of plasma in EDTA, rather than ACD preservative. This special collection procedure is costly in loss of cells and reduced yields of albumin

(Kistler 1971, personal communication). On the other hand the use of ion-exchange cellulose (Smith and Robinson 1971) allows utilisation of ACD plasma, from which AHG is recovered prior to absorption; (for the present) provides a concentrate containing factors II, IX and X at concentrations greater than achieved by the alternative system. This system also recovers approximately twice as much activity in the concentrate. Even here secondary alternatives exist, the inclusion of factor VII in this product is possible but carries with it the problem of controlling the formation of activated factor X (Xa). Unfortunately, decision and choice in this area is clouded because of lack of objective evidence of the need for factor VII in any but the most rare of clinical situations, where this deficiency is genetically determined. It might be easier to identify these cases of four-factor deficiency in which diminished factor VII activity is a true limiting factor in haemostasis rather than to develop a new, high-yielding concentrate containing factor VII activity to replace PPSB. Limited production of PPSB could then be reserved for those situations where it is essential.

4. HEPATITIS

One of the evergreen conundrums of plasma fractionation is the early discovery that the immunoglobulin-containing fraction II, unlike preparations of fibrinogen and AHG, does not appear to transmit serum hepatitis. It has subsequently become fairly clear that, despite conflicting evidence (Grossman, Stewart and Stokes 1945; Mirik, Ward and McCollum 1965), immunoglobulin cannot be expected to confer any real protection against serum hepatitis (Duncan, Christian, Stokes, Rexer, Nicolson and Edgar 1947; Stokes, Blanchard, Neefe, Gellis and Wade 1948: Holland, Robinson, Marrow and Schmidt 1966).

The identification of the hepatitis associated antigen (HAA) in 1967 (Blumberg, Gerstley, Hangerford, London and Sutnick 1967; Prince 1968) and the very rapid increase in knowledge of this entity have not exactly clarified the situation.

A screening programme which results in identification of HAA carriers among blood donors, even if such identification be less than totally accurate, is bound to reduce the incidence of infection in recipients of whole blood, cellular components and whole plasma. However, it is equally certain that such screen procedures, unless they be absolutely infallible, will not greatly influence the infectivity of plasma products. This must remain the province of the fractionator and the characteristics of his technology until such time as screening systems are capable of identifying HAA presence in dilutions at least six orders of magnitude greater than can presently be detected. This, at least, is clear from the much publicised work at Willowbrook School. Many commercial fractionators and some state organisations process pools containing as many as 30 000 donations of plasma; one unidentified infected donation would be enough to make the whole of such a pool suspect.

The fraction II+III, precursor of fraction II which yields immunoglobulin, is known to be one of the most contaminated fractions when HAA-positive plasma is fractionated (Schroeder and Mozen 1970). The fraction II, while in the crude paste stage, contains at least 50 per cent. of its mass as liquid supernatant which has also been in contact with the paste of fraction III and was used originally to dissolve the contaminated fraction II+III. Why then has immunoglobulin (fraction II) administration never resulted in overt disease in recipients? The most probable explanation is

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that in any pool is included some HAA antibody which can render the agent non-infective, possibly during the processing of fraction II + III. Fraction II is lyophilised to remove alcoholic residues in machines which also are used to dry such fractions as fibrinogen and AHG concentrate; both notoriously icterogenic. One would imagine that, at some time in at least one instance, a batch of fraction II would have become cross-infected. Thus it seems even more likely that the HAA freedom of immuno-globulin preparations can only be due to an inherent character of the product; in fact to a fortunate accident. Presumably the neutralising agent is present in such small dilution as to have no detectable effect when normal immunoglobulin is admin-istered for prophylaxis against HAA (Holland, Robinson, Morrow and Schmidt 1966).

Screening procedures, applied to normal immunoglobulin batches, have not detected anti-HAA activity in our products. Very sensitive procedures are required to detect this antibody in normal fraction II preparations. On the other hand antibody detection has successfully identified blood donors who carry significant quantities of this antibody and several fractionators, including both UK centres, have prepared a specific anti-HAA antibody which has been used for prophylaxis with apparent success in at least 32 cases of high-risk laboratory accidents. These specific prepara-tions, from plasma of known antibody status, do provide convincing *in vitro* evidence of anti-HAA activity.

Great caution must be exercised in the search for carriers of anti-HAA activity because the routine abstraction of the plasma of such donors from the pools of plasma processed to provide normal and specific immunoglobulins (other than anti-HAA) could result in the production of a batch or batches lacking the inherent safety factor upon which we are totally reliant. The risk is slight because, presumably, just as screening fails to detect all HAA carriers so will it fail to detect all carriers of antibody.

However, until much more is known about this characteristic of our products, it would be prudent to identify all donations of plasma, screened for anti-HAA content and found lacking, and to consider exclusion of these from process pools destined to provide immunoglobulin or, alternatively, include them as a minority component in a pool of unscreened plasma.

5. DEVELOPMENT OF IMMUNOGLOBULIN PREPARATION

The Cohn fraction II preparation is composed almost exclusively of the IgG immunoglobulin cohort although different fractionation methods will include small varying amounts of IgA and IgM. The antibodies contained in the fraction are, broadly speaking, those to be found in the donor plasma although there may be relative concentration variation in some instances. One batch of non-specific immunoglobulin was found to contain at least 20 of the commoner antibodies (Schwick 1970). These included antibodies against diphtheria, tetanus, typhoid, coliform, varicella, herpes, rubella, measles, poliomyelitis, mumps, influenza and smallpox. Most of these antibodies are normally present in quite low concentrations so that, where specific activity is required, it has been usual to select plasma from hyperimmunised or convalescent donors to produce fractions containing that activity in higher than normal concentration. This has been successfully applied for preparation of a wide range of products in many parts of the world. The Edinburgh Centre now routinely processes

plasma to produce tetanus antitoxin, anti-vaccinia and anti-rhesus immunoglobulins although only the last meets the national needs. The United Kingdom is one of the few Western countries in which tetanus antitoxin is still supplied mainly by heterologous antisera.

The fractions now available are considered to have serious disadvantages in that they contain aggregated polymers of the original protein which makes them unsuitable for intravenous administration (Schultze and Schwick 1962) because of anti-complementary activity and they are unstable during storage. This instability can be modified slightly by adding a variety of amino acids to the final preparation and by avoiding low protein concentrations. It would appear that the mechanisms of polymerisation and fragmentation are interrelated (James, Henney and Stanworth 1964) and it can be shown that this is influenced by the source and method of preparation of the fraction (Sgouris 1970; Škvařil 1970). Administration of such fractions may result in antibody formation in response to their altered antigenicity (Stiehm and Fudenberg 1965; Henney and Ellis 1968; Ellis and Henney 1969). Also the efficacy of the product can be altered by fragmentation (Adam and Škvařil 1965) but it is difficult to assess the significance of this since it is largely dependent on the dose administered and the amount of antibody necessary to afford protection in any particular instance. One cause of fragmentation is suggested to be the presence of small amounts of plasmin in the product (Connel and Painter 1966; Painter 1967).

Limited investigation of Scottish fraction II preparations demonstrates the presence of polymer in the recovered paste, increased polymer after lyophilisation and a further steady polymer formation during storage at 4°C. No evidence of fragmentation has been noted, despite careful analysis by the methods used by Painter and Škvařil. Discussion among fractionators leads to the suspicion that fragmentation is much less noticeable in the products of European fractionators and that this is probably a result of the different methods of handling, storing and fractionating plasma.

At this stage in our development it would be unwise, due to lack of data, to ascribe cause and effect but it is of interest that a considerable number of patients receive regular intravenous administrations with 5–10 g of immunoglobulin in prophylaxis of antibody deficiency syndromes. As expected (Gitlin and Janeway 1956; MRC Report 1969) these patients have responded well to the administration, although none are the types most likely to show reaction to the intravenous material (Schultze and Schwick 1962).

The obvious advantages of an intravenous immunoglobulin preparation are so great that much effort has been expended on development (Barandun, Kistler, Jennet Isliker 1962; Schiff, Sutherland and Lane 1968; Crain, Rosen and Janeway 1970; Schwick 1970; Sgouris 1970), which has mainly taken the form of producing deliberate fragmentation by controlled action of proteolytic enzymes. The half-life of several of these products in the circulation is so short (4–6 days) that they are not to be encouraged for prophylactic repair of deficiency syndromes (Schwick 1970) which is unfortunate when one considers that such situations are, perhaps, those most clearly in need of large amounts, more easily administered intravenously.

The preparation of highly concentrated and specific anti-Rhesus immunoglobulin (Hoppe, Krebs, Mester and Hennig 1967) using ion-exchange cellulose separative techniques has allowed the introduction of routine intravenous therapy in Germany and in Ireland but this product is dependent on the specially cathodic nature of the

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particular antibody (Frame and Mollison 1969) and on the availability of especially high concentrations of antibody in the original plasma. Cohn fraction II, made from plasma of similar titre, would be even safer than the Hoppe product for intravenous use but the clinical advantage would be slight beyond the fact that only half the amount of antibody would be required to induce a particular clinical response. Analysis of data from the application of Hoppe's method in Ireland (O'Riordan 1971, personal communication) shows clearly that the major contribution, to high concentration of the antibody in the final product, is afforded by the highly successful method of selecting and hyperimmunising donors.

Since only a small donor population is required to supply plasma for production of specific immunoglobulin by the ion-exchange method it would appear to be a relatively simple matter to ensure that none are carriers of HAA antigen. However, such is the doubt of fractionators and their faith in organic solvent systems that it is usual to include a stage similar to the fraction II precipitation using a conventional system. This results in huge losses of antibody at a stage when it is already fairly concentrated. These losses, up to 40 per cent. of the original antibody, seem a high price to pay for doubtful insurance against hepatitis.

The cathodic nature of IgG and especially that of certain antibodies can also be employed in electrophoretic fractionation systems (Fleetwood and Milne 1968; Watt 1970*b*) but this method, whereas it can concentrate anti-D antibody in preference to the bulk of IgG molecules, still requires a great deal of development to become an effective tool for routine use. Incentive to carry out such development may well be found in the suggestion that immunoglobulin, separated by electrophoresis, is more resistant to aggregation (Bier 1971, personal communication). A secondary benefit of application of electrophoresis is that a broad fraction can be obtained which contains significant amounts of IgA and IgM as well as IgG (Cosine 1971). This fraction has been further separated by column chromatography to provide a fraction of pure IgM.

The preparation of immunoglobulin fractions containing IgA and IgM is a matter of great importance; Schwick, Fischer and Geiger (1970) have prepared two fractions, one containing 20 per cent. IgA and the other 20 per cent. IgM as enrichment of basic IgG fractions. Clinical assessment of these products in young infants and the elderly are in progress and results are reported as satisfactory (Schwick 1972).

Although conventionally prepared non-specific immunoglobulin contains antibody against rubella it has been singularly unsuccessfully used in preventing foetal damage in the case of women exposed during pregnancy (MacRae, Mogford and Reid 1970). This is an example of a situation which cannot be usefully continued. Non-specific immunoglobulin is rarely assayed to discover the content of antibodies. Various batches, examined to discover their content of ten antibodies (Mosely and Brachatt 1970), showed rubella antibody as high as 1/1280 and as low as 1/160. Since the product is usually prescribed by volume (or weight of protein) it is evident that the degree of protection afforded by one batch would be likely to be ten times greater than by another. It seems logical to suggest that, to provide clinical guidance, it would be better to assay the biological activity of immunoglobulin and, as far as possible, compound the clinical unit as near a normal standard as possible. This could be achieved by selection of batches of powder for mixing to provide the final clinical product. Alternatively, the development of affinity chromatography (Cuatrecasas and

Parikh 1972) may provide a means to recover the more desired antibodies from non-specific batches of material to provide specific immunoglobulins.

Search of normal donor populations for carriers of specific antibodies in high concentration and selection of such plasma into special processing pools can be highly rewarding (Levine, Wyman and McComb 1967; Krijnen, Brummelhuis and Beentjes 1970). Anti-vaccinia, tetanus antitoxin and anti-rubella immunoglobulins from such a source can provide good-quality products. Sixty plasma donations, from donors recently re-immunised with tetanus toxoid had plasma antitoxin levels ranging from 0·2 to 50 units/ml but with an average of 5·5 units/ml. Of these donations, 15 yielded 68 per cent. of the total antibody available for recovery. Thus, selection on a basis of recent immunisation is not entirely adequate for ensuring a good-quality product.

6. Yield and Quality of Plasma Fractions

These factors are largely interdependent and it is a major task for the fractionator to determine the point at which compromise must occur to provide a clinically acceptable product. The fractions obtained are not absolute and losses occur. Broadly speaking, in the preparation of any protein fraction, about 10 per cent. is lost for each stage in the process leading to its isolation. Thus, in the recovery of crude fraction V paste about 40 per cent. of the albumin in the starting plasma is lost into the other stages. This loss is reduced to about 30 per cent. in the use of crude albumin destined to be finished as PPS because it is not purified to the same extent.

In circumstances where a pool of plasma is being processed with high yields of one particular fraction as the main aim, as is the case when preparing specific immuno-globulin, then this is achieved at the expense of the other fractions. There can be serious consequences if zeal for high yields is carried too far. For example, high-yielding immunoglobulin fractions may contain small amounts of fibrinogen which should have been removed in fractions I and III. The presence of fibrinogen may cause difficulty in final filtration of the immunoglobulin, thus placing additional hazard on the product by infringing sterility. Also, fibrinogen is liable to reduce the storage stability of the product.

Different processes may give very different yields of a particular fraction and this is especially seen in coagulation factor concentrates. The calcium adsorption method for preparing PPSB yields about 30 per cent. of the factor IX activity of the starting plasma whereas the use of ion-exchange systems provides recoveries of 60 per cent. of the activity.

The overall effort of fractionation is geared to recovery of albumin, either in the purest form or as the slightly less pure PPS. Yields vary according to the intensity of effort made toward harvest of the preceding fractions but should be 55–60 per cent. as 98 per cent. albumin or 70 per cent. as PPS. Efforts to increase yields are directed toward means of reducing wasteful stages in processing.

Whilst recovery of crude fractions, as pastes, can be controlled and reproducible there are inescapable losses which occur during sterile filtration, filling final containers and resulting from the scrutiny of quality control on the final product. These losses are minimised by optimising the relationship of batch size and equipment capacity. For example, filter pads become sodden with the liquid being processed; this con-

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stitutes a finite loss of product which is proportionately less when the volume processed approaches the capacity of the filter.

7. FUTURE CONSIDERATIONS

The technology for preparing plasma fractions for clinical use has been relatively static for many years and there has been a general reluctance to change. However, the changing clinical demand pattern is forcing a need for diversity in processing technology. Acceptance of the inherent risk of some of the newer fractions calculated against immediate patient benefit has opened new avenues for such diversification. In most countries the need for new fractions is less than the need for more abundant supply of these now available. However, the decision of whether or not to apply the newer technology, and even the decision regarding the choice and composition of new fractions is not easily resolved.

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