PRODUCTS OF HUMAN BLOOD*

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Although blood in its entirety is often irreplaceable, each of its components has its own therapeutic function and one means of economising in the use of human blood consists of separating these and using each individually. Mechanical separation of plasma from cellular components presents no difficulties, but the protein constituents, albumin in particular, can be separated only by a methodical fractioning process like that which has been elaborated and progressively improved by the Harvard School since 1943[1].

This brief article will add little to the excellent literature recently published[2] on blood, plasma and human serum, except to add some new technical facts which are also applicable to the derivatives of blood.

Cell Constituents of the Blood

Erythrocytes, leucocytes and platelets, although they are distinct in nature and functions, have one characteristic in common—their fragility —which, up to the present, has limited their use by injection for therapeutic purposes.

Erythrocytes. The red blood cell is short-lived and fragile in nature. Its preservation outside the body is therefore difficult because of the artificial conditions to which it is subjected. To prevent the blood from coagulating, citrate, itself an abnormal constituent, is added and the red corpuscles are kept inactive and at a low temperature in the container in which they have been collected. Their metabolic rate and gaseous exchanges decrease but glycolysis persists for some time and the lactic acid which is formed reduces the pH below the physiological level. The red cell loses potassium, gains sodium and water, and the resistance of the membrane gradually becomes less.

It has been found that a mixture of citric acid, citrate and dextrose favoured the survival of erythrocytes. The citrate, which cannot pass across the membrane of the cell, exerts an osmotic pressure which counterbalances the endocellular osmotic pressure, and in this way retards hæmolysis. The dextrose allows the cell to continue its metabolic process, while citric acid acts by lowering the pH, which is known to facilitate survival. The alteration in nature of the preserved cell is not, however, due only to the internal hypertonicity: even under physiological conditions the red corpuscle finally disintegrates.

At the present time the citric acid-citrate-dextrose mixture is widely-used as an anticoagulant in the U.S.A,[3], especially when it is desired to preserve the red cells. Since low temperatures (between + 4° and + 6°C.) stop metabolism the cells are more easily preserved. Several attempts have been made to keep them at an even lower temperature, but the thawing process destroyed them. However, recent tests, in the presence

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of glycerol, are quite promising: 70 per cent. of red cells preserved at −79° C. for a period of 6 months in a 30 per cent. glycerol solution, together with 3 per cent. of trisodium citrate, have been found to survive. These conditions cannot be achieved in practice, but the technique is improving to an extent that it is now possible to envisage preservation for a period of three months at −20° C. in a medium containing citrate and a large proportion of glycerol. When the medium is returned to a normal temperature the glycerol may be removed by continuous washing of the red corpuscles in increasingly diluted solutions of glycerol, the operation taking two hours[4]. This process is still only in the experimental stage. However, transfusion laboratories have already been successful in preserving erythrocytes of rare blood groups by freezing them in a medium containing glycerol.

Plasma was employed on a large scale during the war, while the erythrocytes remained unused. This situation has changed to-day, however. The B.P. 1953 devotes a monograph to them which says they may be obtained from one or more preparations of Whole Human Blood which is not more than seven days old and each of which has been directly matched with blood of the intended recipient. The Concentrated Human Red Corpuscles must be used within 24 hours of their separation. Whether they are in the form of a puree or in suspension in an isotonic solution, their intravenous injection should be accompanied by the same precautions as are usual for whole blood. They replace freshly-drawn blood when corpuscular regeneration only is intended. Furthermore, they have more specialised applications: e.g., they are preferable to whole blood in those patients who are likely to overtax their circulatory system; they are prescribed exclusively in hæmolytic jaundice suffered by patients who have undergone a series of transfusions[5].

The red corpuscles are also prescribed for external use, in ointment or powder form, as energetic stimulants to the healing process. For this purpose, both human and animal blood has been used. Lehmann[6] has provided a formula for blood paste using fresh human blood, drawn aseptically, and citrated, to which an agar emulsion is added. It is necessary to obtain an adhesive preparation which remains moist and homogeneous and for this purpose, mucilage of agar appears to be more suitable than carbowax or polyethylene glycol. Vehicles containing greases or fatty alcohols are unsuitable.

Leucocytes[7] *and Platelets*[5,8]. The transfusion of leucocytes and platelets is still in the experimental stage. Leucocytes are rich in enzymes, and their phagocytocic properties, and the part played by certain of them in the formation and transportation of antibodies, makes them of particular interest. However, they are difficult to separate and to preserve, both because they are short-lived and because these cells are rendered fragile by virtue of their greatly hydrated protoplasm within an adhesive membrane. When they are separated by the special Cohn centrifugal machine which has the property of moistening the membrane, they are preserved in special media with a pH value of 7 with monovalent crystalloids and colloids without albumin but containing gelatin, traces

of ascorbic acid, a small quantity of sodium acetate and traces of deoxyribonuclein. Darkness is essential and the optimum temperature is + 4° C[7,9]. In spite of these precautions, not only is preservation short-lived, but when leucocytes are injected as a cream or in suspension they disappear in less than an hour and reappear fixed in the lung. It is, however, possible that the long-term effect of such therapy is favourable.

Leucocytes and platelets represent scarcely 0·4 per cent. of the blood as a whole. Of all the cellular constituents in the blood the platelets are the smallest and lightest in weight. Their origin is still much discussed, but recent findings do not seem to contradict the hypothesis that platelets are simple cytoplasmic fragments of the megakaryocyte.

Little is known of their metabolism: it seems that they have no synthetic activity. When injected, they disappear in four or five days and are fixed by the reticulo-endothelial tissue and especially by the spleen, as recent tests with ^{32}P have verified[10].

During the coagulation of the blood, the platelets play a large part in the formation of the thromboplastin and contractions of clots. On removal from the vessel, it appears that the platelets are combined with calcium; this conception makes it at least possible to understand how easily the platelets may be fixed by the cation exchange resins used to remove calcium from the blood. The blood is then incapable of coagulation. The platelets are then eluted by a large volume of saline solution or a very small quantity of a solution of sodium or calcium acetate, which implies that the calcium is associated with the platelet lipo-protein, i.e., with the thromboplastin. This process combined with centrifuging, by taking advantage of the low density factor of the platelets in silicone or plastic coated containers, has refuted the idea that platelets are fragile and immediately destroyed on removal from the blood vessels.

Protein Constituents of the Plasma

Albumin and globulins were first of all separated by the "salting-out" method, i.e., by fractional precipitation with concentrated acid salts or neutral salts. However, the distinction between euglobulins and pseudo-globulins, defined by the ammonium sulphate concentration which precipitates them, is not apparent in other tests, in particular, on electrophoresis, which separates four principal fractions of the plasma proteins, namely, albumin and α-, β- and γ-globulins, the albumin being the most mobile and the γ-globulin the least. In the normal man, the average percentage of each fraction in comparison with the total proteins in the serum is as follows[11]:

Albumin 61 $\pm$ 3 per cent., α-globulins 12·5 $\pm$ 2 per cent., β-globulins 12·5 $\pm$ 2 per cent., γ-globulins 14 $\pm$ 2 per cent.

The salting-out process, based on a single comparison test, was unselective and inapplicable in practice to the separation of blood-proteins for therapeutic purposes. During the last war it was necessary to use human blood to the best possible advantage, i.e., without waste and using the specific properties of each fraction. The process methodically worked out by Cohn and his associates at Harvard fulfils these

requirements and is still capable of being improved; to-day it is used in most blood transfusion centres in the world[12]. The five variables (ionic strength, ethanol concentration, pH value, temperature, protein concentration) give the Cohn-Oncley 6–9 method plenty of flexibility and the separated fractions are practically identical with the components revealed by electrophoresis.

Plasma is thus divided into five fractions: fraction I is separated from the citrated plasma at a temperature of $-3°$, pH value of 7·2, ionic strength of 0·14 and an ethanol concentration of 8 per cent., while the protein concentration is 5·6 per cent. This precipitate is essentially made up of fibrinogen and antihæmophilia globulin. The supernatant fluid, when cooled, brought to a pH value of 6·9, ionic strength of 0·9, protein concentration of 3 per cent., then has ethanol added in a sufficient quantity to produce a 25 per cent. concentration. The prothrombin, the γ-globulins, the *iso*-agglutinins, the plasminogen and the β-lipo-proteins then precipitate to form fractions II and III. Fractions IV and IV–4 comprise the α-globulins, the α-lipo-proteins and the β-globulins, while fraction V is mainly albumin.

However, the disadvantages of the Cohn method are the cost of installation, the slowness of the operations and the denaturing action of ethanol upon proteins. At present attention is centred upon the use of heavy metals, for example zinc, already suggested by the Cohn 10 method, which is quicker, and in particular by method 12 which acts on whole blood. The metal processes are quick and economical: they save expensive refrigeration plant and their efficiency is greater.

These methods have made it possible to separate a large number of plasma proteins or fractions where a single type of protein predominates: albumin, globulins (α, β and γ), fibrinogen, antihæmophilia-globulins, prothrombin and *iso*-agglutinins. Sometimes the techniques are sufficiently sensitive to produce associated proteins such as α- and β-lipo-proteins. Undoubtedly, the separated fractions are mixtures. They nevertheless lend themselves to therapeutic requirements.

Albumin. This constitutes 99 per cent. of fraction V of Cohn-Oncley method 6–9, but the stable plasma protein solution which Cohn obtains after extracting the α- and β-globulins and fibrinogen from plasma by the zinc method is particularly rich in albumin. Albumin plays a multiple role in plasma: it fixes and ensures the movement of numerous bodies, it contributes to nitrogenous nutrition but, in particular, its high colloidal osmotic pressure causes it to play an important part in the regulation of the hydration of the organism. Not only does it retain the fluid in the blood vessels (1 g. of albumin retains 18 ml. of fluid), but it absorbs the extra-cellular fluids at capillary level and thus prevents œdema[13,14].

However, albumin is able to transmit the virus of serum hepatitis, and an effort has therefore been made to destroy the virus without denaturing the albumin. The addition of acetyltryptophan 0·04 M and sodium caprylate 0·02 M makes it possible to heat a solution of albumin

to 64° C. at pH 7·1 to 7·3 for several hours without change[15]. Ultra-violet light may be used to sterilise the plasma if sodium caprylate is added to prevent the denaturation of the albumin and the other proteins[16].

In practice, the albumin used in therapy is available in a 25 per cent. solution in 100 ml. bottles, one bottle constituting one dose. This solution, at pH 6·8, is stabilised by tryptophan, or by 0·2 M sodium caprylate or mandelate.

The test for the official solution consists of identifying the albumin by its mobility when subjected to electrophoresis, and by checking clarity when the solution is heated to 57° C. This solution should be sterile, apyrogenic and atoxic to mice. It is stored at a low temperature.

Albumin is used principally in the treatment of hæmorrhagic shock conditions where it is of paramount importance to re-establish the blood volume: 100 ml. of a 25 per cent. solution has the osmotic value of 500 ml. of blood. The injection is followed by a blood-dilution of extra-vascular origin. If repeated injections of albumin are given or if there has been considerable hæmorrhage, the injection should be followed by an injection of isotonic fluid, and in serious cases it is used only as a temporary measure until such time as a whole blood transfusion can be given, the latter alone being capable of combatting anoxia.

Another use is in the treatment of serious burns where fluid is constantly leaking from denatured capillaries; here the albumin solution is diluted to 5 per cent. with isotonic saline. A further application is in the treatment of hypo-proteinæmia where the protein deficiency is primarily an albumin deficiency. Injection of albumin is prescribed in the so-called deficiency œdema, in cirrhosis and in nephrosis. The dose, given by perfusion, is 50 to 75 g. daily for an adult and 25 g. for a child. However, repeated injection may lead to anæmia or to hypoprothrombinæmia[14], while the use of large quantities or too rapid injection may create the risk of pulmonary œdema.

γ-Globulins. So many papers have been published recently on the γ-globulins[12,17,18] that no attempt will be made here either to give a definition of them (wholly electrophoretic), nor to describe their venous or placentary origin[19] leading to products of the same immunological properties[20], nor their extraction where the metal techniques[12,18,21,22] are slowly superseding the Cohn-Oncley process, nor their physical, chemical or biological nature[17,23,24]. Their entire interest lies in their antibody properties. It is due to the fact that his blood is rich in γ-globulins that the newly-born infant owes his immunity to disease during the first few months of life. In the same way, passive immunity—which is more durable in the child than the adult—corresponds to a longer average life of the injected γ-globulins and, for a given age, acquired immunity also is longer or shorter according to whether the receiver is able or not to re-use the specific functional groups with their antigenic properties.

We shall confine ourselves solely to the question of presentation of and test for the γ-globulins.

The γ-globulins when prepared give a pale yellow or brownish solution because of the presence of denatured hæmoglobin which is clear or

slightly opalescent and which may, in the course of time, leave a granular deposit. In the United States, doses are generally indicated in ml. of a 16 per cent. solution. In France, Ardry recommends a 10 per cent. solution to be prescribed in mg. of active product[12]. Antiseptics such as sodium ethylmercurithiosalicylate at 1 : 10,000 may be added to these solutions as well as a stabiliser like tryptophan, glycine, alkaline salts or sodium thiosulphate. The final solution is neutral and isotonic.

The tests are designed for inclusion in the French Pharmacopœia[25] and comprise:

1. The general tests for injectable solutions: neutrality (pH value $6{\cdot}8 \pm 0{\cdot}2$), sterility (in a thioglycolate medium), and toxicity to mice.

2. Identification and purity tests. Identification by immunological reactions (the solutions must precipitate with human anti-sera but not with animal anti-sera). Establishment of purity by electrophoretic mobility (90 per cent. of the proteins should satisfy the mobility test $-2{\cdot}8 \times 10^{-5}$) or by precipitation with ammonium sulphate at 36 per cent. saturation (the solution of proteins having previously been diluted 1 : 10 with normal saline). Under the same conditions the solution of γ-globulins must not give a precipitate with ammonium sulphate at 25 per cent. saturation (freedom from fibrinogen) while 70 per cent. of the proteins should precipitate when ammonium sulphate to 33 per cent. saturation is added.

3. Quantitative tests:

(*a*) measurement of proteins by a colorimetric or gravimetric method.

(*d*) determination of activity: for lack of anything better, standard diphtheria toxin is used. The latter, if injected alone intradermally into a guinea-pig, produces necrosis at the level of the injection site. Mix 1 ml. of a standard toxin solution with 1 ml. of one series of dilutions of γ-globulins to be titrated, and after a period of 2 hours at 37° C. and 22 hours in the refrigerator, inject 0·1 ml. of each of the mixtures into guinea-pigs weighing about 250 g. The neutral mixture should not, after 4 days, have produced necrosis at the injection site. The test for specific γ-globulins taken from hyper-immunised human serum consists of the measurement of their specific efficacy. For example, in the case of immune poliomyelitis globulin, mice inoculated with the Lansing strain are used[26].

It is impossible to give the therapeutic uses of the γ-globulins[27] in detail here. These γ-globulins, which were prepared first of all in the U.S.A. for the prevention of poliomyelitis, in preference to plasma, which is capable of transmitting homologous jaundice[28], have had a considerable vogue. It has since been found that they give only a belated and brief protection and their use, which is hazardous, is too costly[29]. Moreover, the γ-globulins are at present reserved particularly for prophylaxis in measles and in treatment by its attenuation[30,32–34], and for the prophylaxis of epidemic hepatitis[27,35]. However, specific γ-globulins are recommended in the corresponding diseases of, for example, poliomyelitis and whooping-cough.

γ-Globulins are also a major indication of a very different complaint, namely a-γ-globulinæmia, which was discovered by serum electrophoresis and which manifests itself in an extraordinary susceptibility in certain individuals to infectious diseases[36].

The γ-globulins are administered either subcutaneously or intramuscularly. The average dose in the prophylaxis of measles is given as 33 mg./kg. and 8 to 10 mg./kg. in attenuation. In the case of a-γ-globulinæmia a dose of 100 mg. is given twice monthly.

Other blood proteins used in therapy are all connected with the blood-clotting processes[2,7,14,37].

It is known that hæmophilia may be related to the absence of certain factors. Up to the present, only the antihæmophilia globulin has been used in therapy and in the form of fraction I of the Cohn method[38], but it can be obtained in a purer form[39] as an amorphous white substance obtained by lyophilisation. It is available in ampoules containing 200 mg. and is given intravenously.

Thrombin and Fibrinogen. Thrombin and fibrinogen play an essential part in blood clotting and are of great service to therapy.

Prothrombin, which was first isolated from bovine plasma may also be extracted from human blood and transformed into thrombin by the addition of placentary thromboplastin[13]. The B.P. 1953 describes a thrombin of human origin satisfying various tests: solubility in saline solutions; coagulation of citrated plasma in the absence of ionised calcium; sterility and finally activity tests. Thrombin in the United States Pharmacopeia is of bovine origin; it is measured in N.I.H. units[40]. Thrombin is used alone as a hæmostatic in buffered solution or orally[41] in hæmatemesis[42] or by injection in hæmophilic articular hæmorrhages[43], or is used in association with fibrinogen.

The B.P. describes human fibrinogen as a white powder or a friable solid. When placed in a saline solution it may coagulate spontaneously in the course of time but will coagulate immediately if thrombin is added. Its nitrogen content is between 15 and 16 per cent. 85 per cent. of this nitrogen is retained in the clot resulting from the addition of thrombin. Fibrinogen may be identified by electrophoresis (in an aqueous buffer solution of pH 8, mobility is between that of the β and the γ-globulins) and by coagulation when thrombin is added to a freshly prepared solution in saline solution. The preparation should be used immediately after reconstitution.

Fibrinogen, with the addition of thrombin at the time of use, has been widely used in America in neuro-surgery[14,37], in the treatment of burns, and in cutaneous grafting. The use of fibrin clot, formed *in situ* in operations to remove and extract stones in the kidney, is also known.

Fibrin may be obtained by the action of thrombin on fibrinogen under suitable conditions, e.g., fibrin foam, listed in the B.P. as a hæmostatic artificial sponge. Fibrin films or plates of varied shape and consistency are also used, especially in neuro-surgery. These latter preparations should fulfil special tests corresponding to the purpose for which they are required, namely, the absence of toxicity to the tissues, the possibility

of becoming flexible when in contact with salt water, the re-absorption in contact with the tissues in a given time.

NOTES

The practice of blood transfusion, for a long time regarded with apprehension and as a last resort, was developed under the pressure of the needs created by the war. Now, however, a few figures will give an idea of the importance of the products of human blood. In 1953, the National Blood Service[44] issued 2000 ampoules of fibrinogen, 3695 of thrombin and 6545 of γ-globulins. In 1954, the Paris hospitals used 54,000 ampoules of thrombin and 4615 of γ-globulins*. The organisation of the preparation of blood products has been regulated by respective Governments. In Great Britain the Therapeutic Substances Regulations of 1954[45] and in France the law of July 4, 1952 and various decrees and orders for its application[46] have laid down regulations for the collection of human blood and for the separation, fractioning, preservation, inspection and distribution of products from human blood. First importance is attached to asepsis in handling the products and to the absence of any profit motive when the blood is issued.

However, there is still a need for an effort towards accurate standardisation in the direction recently attempted by Ardry with regard to the γ-globulins and, more especially, their checking and control[20].

By degrees, monographs have appeared in the official Pharmacopœias regarding the products of blood. The 1953 B.P. mentions Concentrated Human Red Blood Corpuscles, Human Fibrinogen, Human Fibrin Foam and Human Thrombin, while the U.S.P. XV lists Thrombin, Antihemophilia Globulin and Immune Serum Globulin. A plan is under consideration for including human γ-globulins in the Codex français.

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