ARMY BLOOD TRANSFUSION SERVICE TECHNIQUE FOR THE FILTRATION OF BLOOD PLASMA.

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PRELIMINARY TREATMENT.

For Removal of Fat.—Plasma, having been separated from freshly collected blood and filtered without more than a few hours’ delay, is at first crystal clear. But within a few days it becomes opalescent, this being due to the separation of minute globules of fat. This opalescence can be avoided

FIG. 1.—Pooling of A and B blood.
if the blood is kept at a temperature of 4° to 6° C. for one day, and is there­after warmed to room temperature for six to eight hours before pooling.

For Removal of Agglutinins.—Pooled blood is stood for not less than two hours so that the cells may absorb agglutinins. Blood from A and B donors is pooled into large 2½ gallon bottles with a proportion of B to A of not less than 1 : 16 (fig. 1). Provided the ratio of B to A is not less than this figure most samples contain no agglutinins; a few may contain agglutinins of a titre less than 1 : 8 which is immaterial. The plasma from stored group O blood removed by syphoning may be added to the pool. The lower the concentration of cells in the blood the better will an Alfa-Laval centrifuge (vide infra) separate the plasma.

Separation of Plasma from Cells by Alfa-Laval Centrifuge.

All parts of the Alfa-Laval centrifuge which come into contact with the blood must be sterilized. The delivery outlet is fitted with rubber tubing so that the plasma can be conducted into sterile bottles without opportunity for aerial contamination. The "yield controlling screw" which is fitted at the plasma outlet must be set so that the interfacial layer of plasma and cells is at the minimum distance from the bottom of the cones; with the screw in this position the blocking of the machine with leucocytes is reduced to a minimum.

If separation is begun by running the blood into the empty spinning centrifuge the first yield of plasma contains much haemoglobin; this can be avoided by filling the centrifuge first with saline which is afterwards displaced with blood.

If the plasma becomes tinted with haemoglobin the machine must be taken to pieces, washed with running hot water, re-assembled, filled with saline and the whole process begun again. The Alfa-Laval centrifuge will separate haemoglobin-free plasma from blood up to forty-eight hours old, but older blood yields plasma tinted with haemoglobin, presumably from mechanical action upon fragile cells.

Clarification.

Fat is removed from the plasma by passage through well-packed cotton filters. These are prepared from "Perfecta" pulp which is broken into small pieces and soaked in water in enamel bowls. The soaked pulp is autoclaved at 15 pounds pressure for thirty minutes; this process not only sterilizes but helps to make the texture of the pulp homogenous. The filters are prepared in bottomless Winchester quart bottles, the necks of which are filled with large pebbles (fig. 2); they are sterilized by steaming. Before use the filter is washed through with sterile saline.

The plasma is clarified by suction through these filters into sterile bottles. Each filter will deal with approximately 6 litres after which it becomes saturated. On standing the plasma may become opalescent.
from incomplete separation of the fat during the pre-clarification stages; this opalescence is removed by the sterilizing filtration process (vide infra).

**STERILIZATION.**

This is accomplished by passage through asbestos pads in a Seitz pilot filter.

Asbestos pads possess the property of adsorbing the fibrinogen from the first portion of filtered plasma. When using eight 20 cm. pads the first 500 c.c. of filtrate contains no fibrinogen whilst the next 1,000 c.c. has a very poor content.

The asbestos also removes prothrombin from approximately 10 litres, the amount varying slightly with the batch. Although this 10 litres of filtered plasma contains neither prothrombin nor ionizable calcium most
of it subsequently clots on storage. The rate of clotting is the reverse of the order of filtration. Thus clotting begins in the last bottles within twenty-four hours, but may not occur in the earlier ones for fourteen days or longer or even not at all. If sufficient plasma is passed through the pads (about 15 litres) clotting occurs instantaneously even in the filter. The delayed clotting in filtered plasma occurs more rapidly at 4° to 6° C. than at room temperature.

Clotting in the later batches of filtered plasma is probably due to traces of thrombin, formed on the filtering pad from the activation of adsorbed prothrombin by a metallic ion, probably magnesium, which is able to function in the same way as calcium, and which is known to be present in asbestos in considerable amount.

The prothrombin adsorbed on to the asbestos pad can be removed from the pads by alkali. If, therefore, the pads are washed with N/30 sodium hydroxide at the stage immediately before thrombin begins to appear in the plasma, the delayed clotting of filtered plasma can be prevented.

This is the principle followed in the detailed technique described below.

**STERILIZATION AND ASSEMBLY OF APPARATUS.**

(The letters in the text refer to fig. 3.)

*Filter.*—The Seitz pilot filter is sterilized by steam under pressure. The outlet cock (O), to which is attached a piece of rubber tubing 2 feet long (D), is wrapped in calico and sterilized separately in the autoclave. After sterilization it is connected to the unsterilized filter with the valve closed. Steam is passed into the filter until it escapes freely from all opened valves, whereupon all are closed except the two draining valves and these are so adjusted that any condensed steam can slowly escape. The pressure is allowed to rise to 5 pounds per square inch and is maintained for ten minutes. The outlet valve is then opened and steam is allowed slowly to escape from the attached tubing, the end of which has already been covered with calico. The inlet valve is adjusted so that the pressure is maintained at 5 pounds per square inch for a further ten minutes, after which time the steam is turned off, the outlet valve closed and the apparatus allowed to cool.

*Pooling and Bottling Apparatus.*—For convenience the distributing apparatus is sterilized separately in the autoclave. This apparatus consists of a 4-litre reservoir bottle (C) with a wired-in rubber bung through which is passed two ¼-inch glass tubes. One of these glass tubes reaches to the bottom of the bottle, the other is a short length. The short length is attached to a piece of rubber tubing that leads to an efficient wool filter (W). The long length terminates as a T piece, one arm of which is connected by rubber tubing to two ¼-inch glass tubing delivery pipettes (E and E₁), protected by hoods (F and F₁) made from bottomless Winchester quart bottles. Before use the sterility of these hoods is maintained by large plugs of wool covered with calico held in position by rubber bands.
Technique for the Filtration of Blood Plasma

The other arm of the T piece connects by rubber tubing to a second T piece (G); this is joined to two more T pieces (H and K), and so forms a triangle. One open end of this triangle is connected by rubber tubing to a terminal of short glass tubing (to waste); the other, by means of rubber tubing (L) and a glass connection (M), joins the distributing apparatus to the filtering system. All open ends are protected by calico and wool.
When assembling, the distributing apparatus is joined to the filter by inserting the open glass end (M) into the rubber tubing (D) on the outlet valve with all aseptic precautions. As a final precaution the union is immersed in boiling water for fifteen minutes. Five screw clamps are fitted in the positions 3, 4, 5, 6, 7, as illustrated.

**Positive Pressure Vessel and Alkali or Saline Reservoir.**—The plasma is contained in a tin-lined copper pressure chamber (A), which connects by rubber tubing to the inlet valve (I) of the filter. None of the apparatus to the left of the filter (see fig. 3) is subjected to full systematic sterilization, but is kept scrupulously clean and is assembled after thorough rinsing with hot water. Sterilization of this part of the apparatus is unnecessary because all the plasma therefrom is subjected to filtration. Immediately in front of the filter inlet valve (I) a T piece is inserted. This provides the inlet for the alkali and the saline wherewith the filter pads are washed between successive filtrations; it connects by rubber tubing to a long piece of glass tube (N) that passes through a two holed rubber bung into a Winchester quart bottle (B). A short piece of glass tubing (P) passes through the other hole of the bung and connects to the pump. Two screw clamps are fitted in the positions 1 and 2 as illustrated.

**Blood Transfusion Bottles.**—Pint blood transfusion bottles in which the filtered plasma is collected are plugged with wool covered with gauze and the plug as well as the neck of the bottle are then covered with cellophane in order to prevent dust settling on the rim. These are autoclaved. These bottles are finally sealed with rubber bungs which undergo boiling in a water bath at the time of filtration; this water bath contains distilled water with 1 per cent phenol. The bungs are kept covered with the boiling solution and any loss of fluid due to evaporation must be replaced with the phenol solution. The phenol ensures that the potential space between the rubber bung and the neck of the bottle is filled with a film of antiseptic which lessens the risk of bacteria or fungi growing downwards into the plasma.

**TECHNIQUE.**

**Premises and Staff.**—The filtration and bottling of plasma is carried out in a draught- and dust-free room. The floor of the room is kept moist with antiseptic during the whole time it is in use. The atmosphere should be sprayed with a suitable aerial disinfectant such as Euginol Carbinol.

A staff of four is required; all must wear sterile gowns and masks and sterilize their hands with Dettol. Changing of clothes, coats for gowns, etc., within the room is forbidden.

Operator No. 1 takes charge of the plasma container and is responsible for changing bottles of saline and caustic soda and for washing the filter pads between-whiles. Operators Nos. 2 and 3 are responsible for bottling; Operator No. 4 is responsible for placing the bungs in the bottles (fig. 4).
Filtration—First Batch.—The copper container (A) is filled with plasma and, with screw clamps Nos. 2, 3, 5, and 7 closed, Operator No. 1 applies a pressure of 7 pounds per square inch to the container thereby forcing the plasma through the filter into the reservoir bottle (C). For this first batch, 3 pints only are allowed to run into the reservoir, whereupon clamp No. 1 is closed and clamp No. 2 opened, pressure now being applied through the saline and alkali container (B), which at this stage must be empty. By applying pressure in this manner the residue of plasma in the filter is forced into the reservoir bottle; and pressure is maintained until air begins to appear from the filter. The reservoir now contains 4 pints, which is the largest amount of plasma that can be filtered during this first stage without subsequent clotting.

Fig. 4.—Filtration and bottling team at work.

Bottling.—Clamps Nos. 4 and 6 are closed whilst No. 7 is opened so that the plasma can now be distributed into bottles. Operators Nos. 3 and 4 each work a hooded pipette and each pipette is used alternately. A pressure of 2 pounds per square inch is applied to the reservoir to force the plasma into the pipette. A bottle is placed very carefully under a hood and when removing the plugs care is taken not to touch the rim of the bottle with the fingers; nor must any plasma be allowed to foul the neck of the bottle during the filling process. The flow is controlled by spring clips and filling is so timed that one of the clips is always open. As each bottle is filled it is taken by Operator No. 4 who carefully avoids slopping the plasma on to the neck of the bottle and who keeps the mouth of the bottle covered with a Bunsen flame from the time it leaves the hood until the bung is pushed into position. Bungs are removed with forceps from the boiling water bath.
containing 1 per cent phenol in distilled water which has previously been described. The top of the bung and the neck of the bottle are swabbed with a watery solution containing 10 per cent glycerine and 2 per cent phenol. The top of the bottle is then covered with a viscap which has previously been soaked for one hour in a watery solution of 1 per cent phenol and 2 per cent glycerine.

**Washing the Filter with Alkali.**—Whilst the filtered plasma is being distributed the filter is being washed with alkali and afterwards cleared with saline before continuing filtration. Clamp No. 3 is opened and 2 litres of N/30 caustic soda, contained in one Winchester bottle (B), are forced through the filter and allowed to run to waste. The Winchester of alkali is then changed for a Winchester of normal saline which passes through the filter and so displaces the alkali. Another Winchester of saline is then connected and after a total of 3 litres has passed, clamps Nos. 4 and 5 are opened thus washing away any alkali which has collected in front of clamp No. 4. The 4 litres of saline are followed by plasma by closing clamp No. 2 and opening No. 1, but the filtrate continues to run to waste until plasma appears. Clamp No. 6 is then opened whilst clamps Nos. 3, 5 and 7 are closed.

**Filtration—Subsequent Batches.**—Eight pints of plasma may now be filtered before the filter is again washed. It is important to shake the reservoir periodically in order to ensure that the plasma is homogeneous. At least 150 pints of plasma can be filtered through the eight plates of the filter, but washing must be carried out after every eighth pint.

**Sterility Tests.**—Sterility tests should be made by running 20 c.c. of plasma into 100 c.c. of 1 per cent glucose broth contained in a pint blood transfusion bottle. Six tests should be made during the filtration of 150 pints. The cultures should be incubated for three days. This medium will grow both aerobes and anaerobes.

The plasma should be stored in a warm room for at least seven days before being used. This serves as an incubation period for any casual contaminant, the growth of which will become visible to the naked eye. The detection of bacteria by this macroscopic method is not easy, but plasma which remains crystal clear can safely be assumed to be sterile. Sterile plasma can become opalescent from minute droplets of fat which have not been removed in the early cooling and warming process; this opalescence can simulate the growth of organisms such as *B. subtilis*. Fibrin occasionally forms as granules and this closely resembles the growths of staphylococcus, streptococcus and diphtheroid bacilli. Most of these pseudo-growths can be recognized with practice, but where there is any doubt it is better to make microscopic examinations of the suspected samples, rejecting the contaminated ones and refiltering the others.

**Yield.**—The plasma yield by this process is a little under 50 per cent of the original blood volume when 100 c.c. of anti-coagulant is added to 440 c.c. of blood. The theoretical yield is 60 per cent.

Four workers take about four hours to filter and bottle 150 pints.