CERTAIN PROPERTIES OF PLASMA WITH A NEW METHOD FOR LARGE SCALE PRODUCTION OF A NON-CLOTTING PRODUCT.

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Citrated plasma is now acknowledged as a safe and efficient protein-containing fluid for blood volume restoration; it is widely used as a blood substitute both in fluid and dried form. But it is an excellent culture medium and can only be rendered safe for use by efficient bacterial filtration. The addition of restraining antiseptics has so many potential disadvantages and has been found to afford so little protection that it is no longer practised. Filtration not only ensures sterility but also renders the plasma clear and thus affords a practical means of checking its fitness for use. Safe filtered plasma is always clear; turbid filtered plasma may be contaminated and should be discarded. Unfiltered plasma is always turbid and cannot be assumed to be sterile unless a sample be cultured, an act which itself gives opportunity for contamination. Seitz asbestos pad filtration offers the only practical method but unfortunately large scale work is associated with post-filtration clotting; this may vary from massive clots soon after filtration to fine clumps of fibrin many weeks later. Provided the plasma is clear, clot formation does not prohibit use but may cause mechanical difficulties during administration and fine aggregates may make the fluid appear turbid when the bottle is inverted. Post-filtration clotting has previously been...
shown (Bushby and Whitby 1941) to be caused by thrombin. Magnesium silicate, the principal ingredient of the asbestos from which the pads are prepared, has the property of absorbing a certain amount of prothrombin. The first portions of plasma which pass through an asbestos filter are therefore devoid of this proenzyme but that which is absorbed on the pad slowly becomes activated to thrombin.

The method devised to eliminate this difficulty (Bushby and Whitby 1941) made use of the fact that prothrombin is soluble in weak alkali. By washing the filter pads with \( \frac{N}{30} \) \( \text{NaOH} \) the prothrombin on the pad is removed before it can be activated.

The method has not been entirely satisfactory; a trace of thrombin is usually formed and this converts small amounts of fibrinogen to fibrin. The formation of visible clots is often delayed because the conversion of fibrinogen to insoluble fibrin is a quantitative process depending upon the concentration of the reagents—fibrinogen and thrombin—as well as upon physical conditions. The change from fibrinogen to fibrin takes place in two stages; the first, a chemical one, in which soluble fibrinogen is converted into soluble fibrin and the second, a physical one, in which soluble fibrin is changed to the insoluble form (Mellanby 1933). The chemical change comes about during filtration by reason of the thrombin formed on the asbestos filter pad. The physical change may be regarded as a process due to aggregation of the molecules. As such it will depend mainly upon the concentration of the fibrin molecules. When the concentration is low several weeks may elapse before visible fibrin is formed; when the concentration is high massive clots appear rapidly. Furthermore the aggregation of low concentrations is accelerated by shaking and also by freezing which has the effect of concentrating molecules in solution. This is the explanation of the delayed clot formation in plasma produced by the alkaline wash process especially when the plasma is refrigerated.

The principle of the present method is to make use of the stability of prothrombin in alkali so that filtration is carried out in the stable phase. In these circumstances no thrombin can be formed and the filtrate is therefore free from the substance that gives rise to post-filtration clotting. Prothrombin is at first retained on the filter pad but is incapable of being activated; it later appears in the filtrate. After filtration the alkalinity is readjusted to pH 7 with \( \text{CO}_2 \).

Method.—The addition of 45 c.c. \( \text{N. NaOH} \) to 1 litre of citrate plasma (100 c.c. 3 per cent sodium citrate to 440 c.c. blood) achieves a pH of approximately 10.6. At this pH if plasma be filtered within an hour or two no post-filtration clotting occurs. Moreover, if the plasma be received into a Winchester quart bottle containing \( \text{CO}_2 \) at 3 lb. pressure, the bottle being agitated during the time of reception, the reaction becomes quickly adjusted to pH 7.

Alkalization of Plasma (fig. 1).—The plasma to be alkalized is contained in bottle A. The suspended transfusion bottle B contains an amount
of N. NaOH sufficient to contribute 45 c.c. to every litre of plasma in bottle A. Suction from a pump, applied through wash bottle C, has the effect of sucking into bottle D the plasma from bottle A and the NaOH from bottle B. Mixture of the two fluids actually occurs at the point X. The inclusion of two control clips Y and Z and the drip counter E enables the speed of flow of the NaOH to be adjusted so that bottle B is emptied at the same relative rate as bottle A. This ensures continuous mixing in

![Alkalization of Plasma](Diagram)

FIG. 1.—Alkalization of Plasma.

the right amount without there being any chance of even a momentary excessive concentration of NaOH.

Filtration and Bottling (fig. 2).—The whole apparatus, with the exception of the CO₂ cylinder and Winchester A containing unfiltered plasma, is autoclaved as a complete unit with all clips open; the bottling hood is plugged with a large cotton wool pad held in place with gauze. The plasma, alkalized as shown in fig. 1, is contained in the Winchester A. The two Winchesters, B and C, are first filled with physiological saline, passed through
the filter with clips K, H, J and L closed. Close clips F and G and open H and J; using 3 lb. pressure CO₂ from container D displace the saline with the gas allowing the fluid to run to waste by releasing clip K. This leaves Winchester B and C filled with CO₂ at 3 lb. pressure. Screw clip F is then opened; all clips other than E are closed. Using 10 lb. pressure of air force the unfiltered plasma from Winchester A through the filter and receive it in Winchester B which must be shaken during reception. When Winchester B is three-quarters full, close clip F and open clip J whereupon the neutralized plasma is forced by the 3 lb. pressure of CO₂ onwards to the bottling hood controlled by clip K. Winchester B when emptied of plasma is therefore filled with CO₂ ready to receive the next filtered batch. At the same time if clip G be opened filtered plasma passes into Winchester C and this in turn, having been well shaken, is ready for bottling by merely closing clip G and opening clip H. The process can thus be repeated as a continuous cycle filling bottles B and C alternately and distributing via the bottling hood by opening and closing the appropriate clips. Winchester A is changed for a full one as and when required. Filtration up to any amount can be carried out without changing the pads but personal experience has fixed the limit at 80 litres in that a single defective pad will ruin a whole batch. The pads tend to collect a certain amount of fat except from especially clarified plasma and it is advisable to wash the filter through with 2 litres N/30 NaOH after filtering each batch of 20 litres; this is done by closing clip E and opening clip L; the NaOH is removed from the pads by washing with 4 litres 0·85 per cent NaCl. When plasma is especially by prefiltration through asbestos pads (for which purpose the filter need not be sterilized) then the pad-washing process can be omitted.

**FIG. 2.—Filtration and bottling.**
Tests for denaturation, toxicity and efficiency of this type of plasma.—The plasma so produced has been fully tested during many months. No more than a summary of the results can be given here. Alkalinization to pH 9.5 for periods as long as six months does not render plasma toxic to animals or human subjects. Indeed, large amounts of such alkaline (unneutralized) plasma have been administered to air raid casualties without immediate reaction or remote effect and with efficient results. Plasma brought to pH of approximately 10.6 by the addition of 45 c.c. N. NaOH per litre does not show evidence of denaturation when examined five days later by the sodium nitro prusside test. It is therefore clear that alkalinization to pH 10.6 for an hour or two has no denaturing effect. Plasma alkalized to pH 10.6, filtered and afterwards adjusted to pH 7 with CO₂ as described above, has been extensively tested on animals and human subjects with very satisfactory results; it can be clotted in a normal manner by the addition of calcium and thrombokinase (lung extract) which implies no denaturation even of prothrombin; nor does it show any evidence of denaturation or alteration of any part of the protein content in that patients who have been transfused with it have, after an interval of three weeks, been tested for dermal sensitivity, followed by a test for general sensitivity to the intravenous injection of 20 c.c. In no case has there been either dermal or pyrexial reaction. From this it is concluded that the transfused protein was in its natural state. Certain other general points are of importance. This non-clotting plasma has been kept for four months under alternating adverse conditions of storage (refrigeration—warming) and has been shaken about in transport without any sign of clot formation (large or fine) and with no deposition. The plasma appears clear when viewed with transmitted light but is slightly opalescent (not turbid) with reflected light; this seems to be due to prothrombin itself. The yield of plasma by this method is approximately 20 per cent more than by the older method with which a great deal was lost during the numerous pad washing processes. The saving in time is very great. The method is readily adapted to filters of 14 c.c. size and if CO₂ is not available neutralization can be effected with citric acid provided it is arranged for mixing of the concentrated acid with the alkaline plasma to occur evenly in a manner similar in principle to that shown in fig. 1.

REFERENCES.