PROBLEMS OF ESTABLISHING A BLOOD BANK

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The following thesis deals with the problems that arose while establishing a blood bank at the Winnipeg General Hospital, and how they were overcome. Problems arising from storage and contamination are dealt with, as well as various investigations into the blood changes occurring during storage. The problem of plasma drying was also investigated. In conclusion a brief outline is offered for the construction and maintaining of a suitable blood bank set up for a 500 bed general hospital.

Blood banks - organized units as such are a comparatively new system of handling indirect blood transfusions. A tremendous amount of literature has been published on this subject in the last five or six years. Blood banks and blood collecting centres (e.g. Canadian Red Cross since onset of present war) have arisen all over the country. Especially in the United States the idea was accepted whole heartedly and investigated most enthusiastically. Although blood banking is comparatively new, use of stored blood, serum and plasma date back many years; as early as 1916 Rous and Turner had published results of their work with stored blood. Robertson[1] and later the Russian workers Skundina, Skindina and Yudin[1] re-aroused interest in stored blood. In America Fantus[2] at the Cook County Hospital in Chicago was, we believe, one of the

first to establish a blood bank. The Johns Hopkins Hospital[3] was also among those who did much of the preliminary work on the problem of banks. In the Winnipeg General Hospital a transfusion of stored blood three days old was used in 1938.

For several years the need for a more adequate blood transfusion service at the Winnipeg General Hospital had been felt. The main objections to the then existing system of calling a blood donor from a donors' list after the doctor decided on a transfusion were:

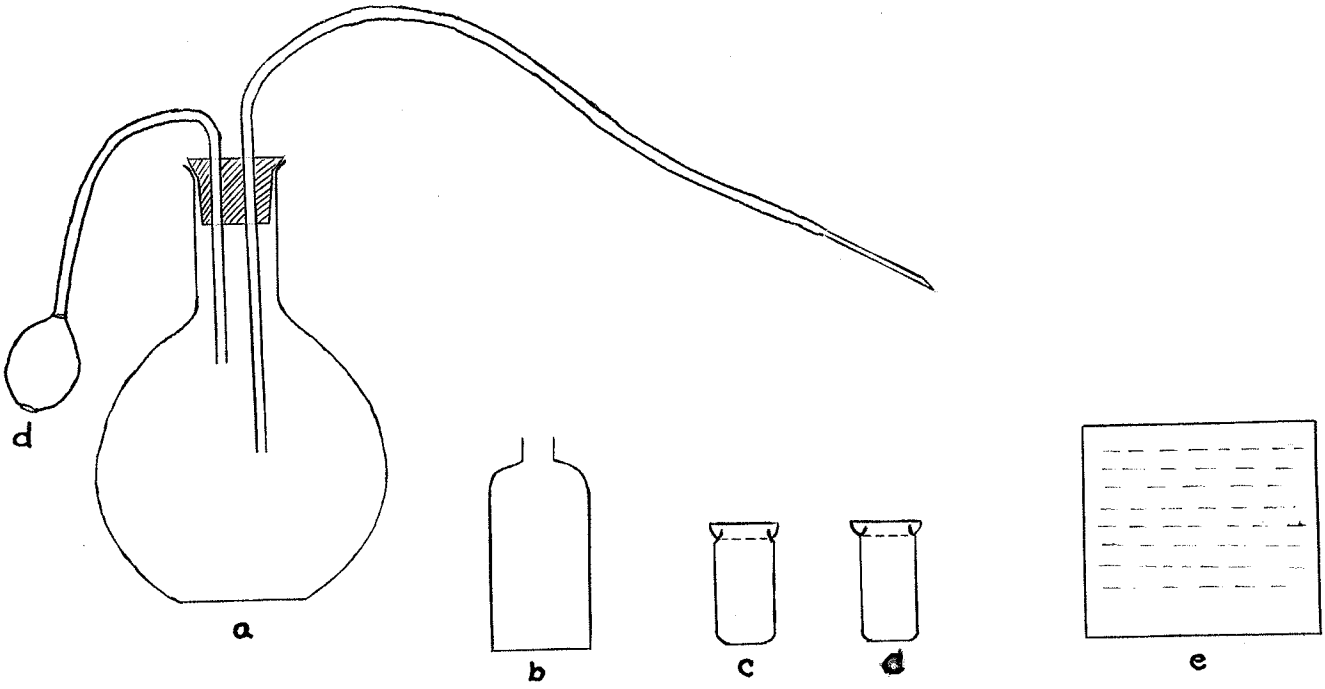
(a) Since the donors were 90% medical students, the summer vacation period produced a definite shortage of available donors, so that often it was impossible to get a group i or group iii donor.

(b) A definite cost to the hospital for those public patients who were unable to pay for the blood they received.

(c) The therapeutic importance of plasma was coming into prominence, and without some method of storage and separation to obtain this plasma it would not be available for use at this hospital.

In June, 1940, the first steps were taken to establish a blood bank at the Winnipeg General Hospital. It was decided that because of the conflicting opinions and reports on the various types of collecting equipment, that we would do our first work with the equipment available and in use in the hospital at that time. With

Figure 1



Showing contents of indirect transfusion bundle first used for the Blood Bank. This includes:
(a) Litre Florence flask
(b) Bottle containing 100 c.c. 2% sodium citrate
(c) 5 c.c. screw-cap bottles
(d) One-way suction bulb
(e) Data card

the assistance of Dr. D. Nicholson and Dr. R. Cooke, the resident in surgery, and using the blood bank of the Johns Hopkins Hospital[3] as a rough guide, the following plans for operation were drawn up.

A. EQUIPMENT

This consisted of: (Fig. 1)

(1) 1 litre Florence flask.

(2) Bottle containing 100 c.c. of 2% sodium citrate.

(3) #7 two-holed rubber stopper containing two short pieces of glass tubing. To one of these pieces of glass a one-way suction bulb was attached and to the other a piece of rubber tubing with a glass adapter at the other end.

(4) Two stainless steel needles #13 and #15.

(5) Two small screw cap glass bottles of 10 c.c. capacity, one of which contained a small amount of dried oxolate.

(6) Data card.

(7) Local set - syringe 5 c.c. size
hypo needle
10 c.c. of 1% novocaine.

(8) Blood pressure cuff.

(9) Alcohol, 2% iodine and sterile drape.

B. SOURCE OF BLOOD

(1) The main source of blood was from friends and relations of the patient receiving the blood. When ever possible two transfusions were requested for each transfusion given. This was found to be necessary to build and maintain a supply since there was always a

certain amount of wastage as well as unpaid accounts.

(2) The other source of blood was from therapeutic phlebotomies: (a) congestive heart failure and (b) polycythemic patients. * The blood from the congestive heart failure donor was considered to be no different than from a normal donor, with the exception of a possible increase in the icterus index of the blood. Therefore, an icterus index was done on all these bloods and if this fell within the normal range of 4 - 6 units the blood was considered safe to use; however, if the icterus index exceeded 10 units the blood was discarded for transfusion purposes.

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* The question was raised whether or not polycythemic blood should be used since at the present time we do not know the nature of the disease. No information could be obtained on this problem since in all the literature this disease, if it may so be called, was accepted as a good source of blood for a bank supply. The question for the moment must remain unanswered. Several polycythemic bloods have been used in the past two years, and no difference between it and normal blood (except for the difference in plasma volume) was noted.

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C. TAKING OF THE BLOOD

1. The pathological laboratory will indicate on the compatibility report the number of blood transfusions, and from whom they are to be taken, for the maintenance of the blood bank. Whenever possible two blood transfusions, or even three transfusions, shall be taken from friends and relations of all public patients receiving a transfusion, when sufficient donors are available.

11. Procedure for Taking the Blood

(a) The blood pressure cuff is applied to the arm; the cubital fossa is then painted with 2% iodine and a small amount of local anaesthetic is injected into the skin. The site to be used is then cleaned with alcohol and the needle is inserted into the vein. The blood is drawn into a Florence flask, into which 100 c.c. of 2% sodium citrate has been drawn. A one-way rubber suction bulb is used to create a negative pressure in the Florence flask. Five hundred c.c. of blood are removed. The rubber tubing connected to the needle is then removed from the flask end, and with the needle still in the vein, the pressure cuff having been released, 5 c.c. of blood is allowed to run into each of two bottles - (1) A small 10 c.c. screw cap glass bottle containing a small amount of oxalate; this is to be labelled "for grouping and compatibility tests." (2) Another 5 - 10 c.c. of blood is allowed to run into a similar bottle, but not containing oxalate; this is to be used for Wassermann reaction and Laughlen precipitation tests for syphilis. The needle is then withdrawn from the vein. The rubber stopper is removed from the flask and the mouth of the flask is covered with several flats of sterile gauze; this in turn is covered with a square 6" x 6" of Venetian silk.

(b) When the blood is taken, the data card found in the transfusion bundle is to be filled in by the interne.

(c) The interne will be responsible for bringing any flasks of blood, which are not to be used immediately, together with the two small bottles and data card to the pathological laboratory, where

they will immediately be placed in the refrigerator.

lll. The patient for whom the blood is taken has the first claim on this blood, and it is not to be released for general use until the third day, unless there are similar compatable groups in the bank.

lV. On the third day the pathological laboratory will ask for a report on the condition of the patient, and if another transfusion is unlikely the blood shall be released for general use.

V. Each week a report will be sent to the different services, stating the blood available in the bank, and it is urged that this blood be used, when it can be done so to advantage.

Vl. At the end of the tenth day the plasma will be removed from the citrated blood and stored.

Vll. At the time of removal of the plasma two cultures will be taken to insure that contamination does not exist.

Vlll. The plasma will be stored for an indefinite period and will be available for plasma transfusions.

D. REMOVAL OF BLOOD FROM THE BANK

1. It must be made clear by the interne or the doctor on the service, to any patient using the bank, that this removal is to be made good by blood from friends or relations.

ll. The blood returned to the bank need not be the same group as that removed, unless the laboratory indicates that there is a shortage in that group.

Figure 2

Blood Bank

The Winnipeg General Hospital

Blood Group.....Titer if "O".....
(AB; A; B; O;)
Signature.....

Donor's Name.....No.....
Address.....Phone No.....
Blood taken for.....
Date blood is taken.....Hour.....
Time of last meal.....
Donor Professional.....or Family.....
Donor's Temperature.....Pulse.....W. R.....
General Nutrition.....
Present ailments (colds, etc.).....
.....
Previous Illnesses.....

Interne's Signature.....

Data card adapted for Blood Bank records.

111. In the case of non-public patients, there is the option of paying for the blood or returning the blood plus a service fee of $5.00.

1V. The oldest blood in the bank must be used first, unless there is some definite contraindication as in agranulocytosis, purpura, and acute infections.

V. Just prior to giving any transfusion a compatibility test will be made between the recipient's blood and that to be given from the bank. The negative Wassermann report will also be stated on the compatibility slip. If plasma is being used, the culture report and its titer will also be stated.

V1. At the time of the giving of a blood transfusion from the bank, 5 c.c. of the blood will be withdrawn and kept for checking any possible reaction or infection that the patient might suffer following the transfusion.

V11. The above rules apply to plasma transfusions as well as to the giving of citrated blood. The titer of the plasma rather than its grouping will be used as a criteria for compatibility.

Fig. #2 illustrates the data card designed and adopted for use at the Winnipeg General Hospital. These cards are filled in by the interne and the blood bank operator and then are filed away for record purposes.

Figure 3

Blood Put in Bank.						Blood Drawn from Bank.						Blood Out-Dated or Spoiled						Plasma Removed from Blood.					
Date	No	Group				Date	No	Group				Date	No	Group				Date	No	Group			
		AB	A	B	O			AB	A	B	O			AB	A	B	O			AB	A	B	O

Double ledger chart for Blood Bank entries and removals.

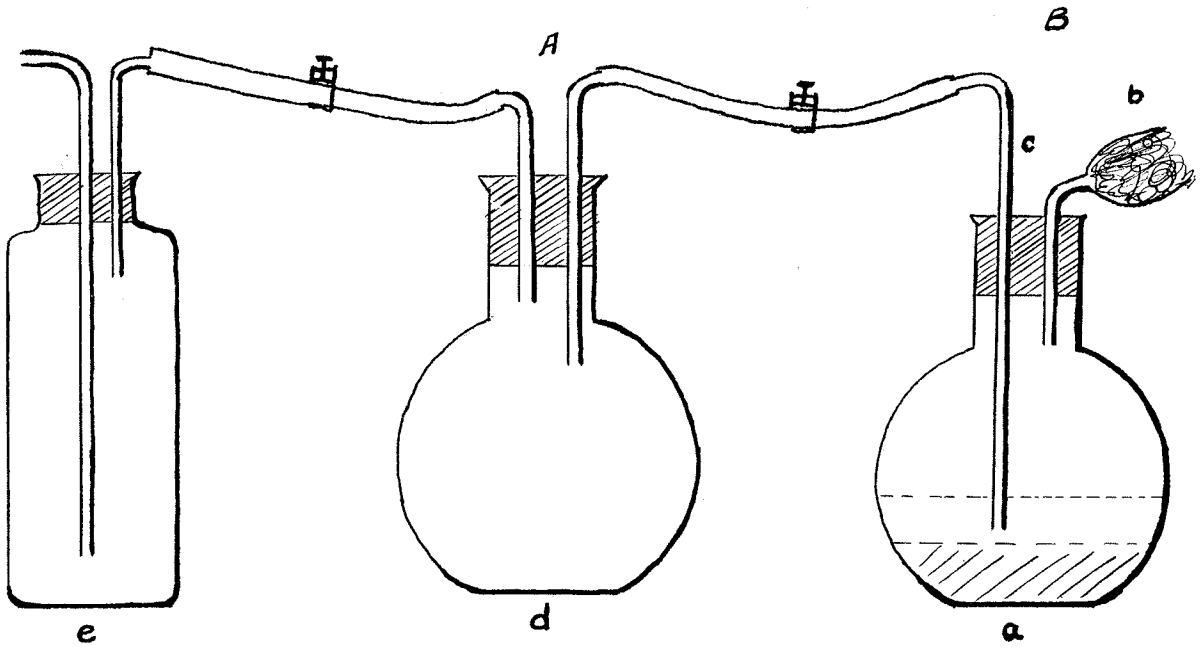
PROCEDURE OF HANDLING THE BLOOD ON ARRIVAL AT THE BANK

Once the blood was received at the laboratory it was immediately placed in the refrigerator at a temperature of 4° - 6° C. Each blood was given a "blood bank number" as well as being labelled by name, a double check against any clerical mistake. All blood and plasmas received into the bank or removed were also entered into a double ledger chart (Fig. #3), so that it was possible to tell at a glance the stock or supply available. A grouping was then done on the sample taken for that purpose, and if the blood was a group IV, Moss, (or Landsteiner group O) a titer was done to determine its universability. A sample of blood was also sent over to the Provincial laboratory for a Wassermann test. Laughlen precipitation test for syphilis was also done on each blood sample at the pathological laboratory. The blood was stored in the refrigerator at 4° C. for ten days; on the tenth day (this has since been reduced to the seventh day) the plasma was removed and the concentrated red blood cells kept for an additional three or four days.

REMOVAL OF PLASMA

The apparatus first used for the removal of plasma was the very simplest, and constructed in the laboratory. The results we obtained with this withdrawal set were fairly satisfactory and the incidence of contamination was very low.

Figure 4



Plasma Removal Apparatus.

(a) Flask containing sedimented blood.
(b) Cotton plug filter.
(c) Glass withdrawal pipette.
(d) Plasma receiving flask.
(e) Water trap.

APPARATUS (shown in Fig. #4)

This apparatus consisted of sections "A" and "B", "A" being a #6 two-holed rubber stopped containing a glass filled "cotton plug" air filter in one hole and a long glass pipette drawn to a fine point in the other. This was connected to section "B" by rubber tubing, which consisted of a similar rubber stopper containing two short pieces of glass tubing, one of which was connected to the water trap "C" and thence to the water suction pump.

METHOD OF OPERATION

This very simple withdrawal apparatus was sterilized in a separate bundle for half an hour at twenty pounds pressure in an autoclave. At the time for removal of the plasma from the blood the blood was removed from the refrigerator, care being taken not to agitate the flask, and placed on the bench. The coverings from the blood flask were then removed and the top of the flask flamed with a burner and section "A" inserted into the flask. Section "B" was inserted into a sterile litre flask, to obtain normal plasma, or into a flask containing 250 c.c. of sterile 0.9% sodium chloride for the production of dilute plasma. A water trap "C" was added to take care of any back-flow. By gently pushing the pipette of section "A" into the plasma and varying the suction pressure, using just enough suction pressure to draw the plasma over, it was possible to remove approximately 35% of the total blood volume. Following the removal of the plasma two cultures were taken from the flask containing the plasma by means of sterile pipettes. The top of the flask was then flamed and covered with several layers of sterile gauze and a six-inch square of Venetian silk. The plasma was then placed in the refrigerator at 4° C.

ALTERATIONS OF THE PRELIMINARY METHODS AND TECHNIQUE

The above described apparatus and technique of handling the stored blood worked fairly well. However, there were several faults which we were interested in correcting:

(a) The above system was an open system, that is, open to the air, and therefore a greater potential source of possible contamination existed than would be present in a completely closed system.

(b) Litre Florence flasks, although ideal for mixing blood, were large and took up too much shelf storage space.

(c) Because of the large surface at the plasma-blood junction the maximum of plasma recovery was only 35% of the total volume, which meant a loss of 1/3 - 1/4 of the plasma.

Efforts were therefore directed along two lines, namely a more suitable system of taking and storing blood, and secondly to reduce the potential contamination.

BLOOD AND PLASMA GERMICIDES

In the literature numerous articles had appeared recommending various chemical reagents to be added to stored blood and plasma to safeguard against contamination, the two most promising ones being the sulphanilamide group[4] and "Merthiolate". Of these two "Merthiolate" seemed to offer the best possibilities. Powell and Jamieson[5] did most of the early work on this compound with regard to germicidal action and animal toxicity. Later Elliott[6] and his co-workers used it extensively in working with stored plasma.

Valuable information was also obtained from Drew, of the Blood Transfusion Betterment Association of New York, by personal communication. "Merthiolate" is the trade name of the sodium salt of ethyl mercurithiosalicylic acid - $C_2H_5HgSC_6H_4COON_a$ - manufactured by Eli Lilly Company. We obtained a sample of "Merthiolate" and made up the following buffered 2% solution:

"Merthiolate" powder.....2.0 grams

Borax.....2.8 grams

Sodium Chloride.....0.85 grams

Distilled water.....to 100 c.c.

A small series of experiments were then carried out to actually determine the germicidal properties this compound possessed. According to Power and Jamieson(5) a final dilution of 1:5000 was an effective germicide as well as being within the safe concentration with regard to toxicity to man; hence in our series we used the 1:5000 dilution.

METHOD

Old, out-dated, but still sterile blood was used. Three sets of 50 c.c. lots of blood in large test tubes were set up. One set was placed at room temperature (20°C.), one set at refrigerator temperature (4° C.), and the third set was placed in the incubator (32°C.). Similar sets of blood not containing "Merthiolate" were used as controls. A variety of bacteria were used to inoculate the blood, including B. Coli, Staphylococcus aureus hemolyticus, Streptococcus hemolyticus, and as well the use of non-sterile containers and air contamination (exposing the open test tube to the air for several hours).

Table 1

	4°C. Refrigerator Temp.		20°C Room Temp.		32°C. Incubator Temp.	
	Merthiolate	Control	Merthiolate	Control	Merthiolate	Control
24 Hours	0	+	0	+	0	0 → +
48 Hours	0	+	+	+	+	+
72 Hours	0	+	+	+	+	+

A

Showing the protective action of "Merthiolate" (dil-1:5000) to streptococcus hemolyticus.

	Refrigerator Temp. 4°C		
	24 Hours	48 Hours	5th Day
Merthiolate	0	0	0 } Strep. Hem.
Control	+	+	+
Merthiolate	+	+	+ } Staph Hem.
Control	+	+	+
Merthiolate	0	0	0 } B. Coli
Control	0	+	+
Merthiolate	0	0	0 } Air Contamination.
Control	+	+	+

B

Showing efficiency of "Merthiolate" as a bacteriostatic agent at 4° C.

The inoculation with bacteria and the addition of the "Merthiolate" were done within two minutes of each other; the cultures were then read at spaced intervals.

RESULTS

The results are shown in table # (1). "A" shows the results obtained following the inoculation with streptococcus hemolyticus at 4° C., 20°C., and 32°C. At 4° C. we got complete protection, whereas at room temperature (20°C.) after the first twenty-four hours the protection afforded by the "Merthiolate" was no longer complete. "B" shows a similar result. From these results it would also seem that "Merthiolate" was a more effective germicide against the streptococcus and B. coli than against the staphylococcus.

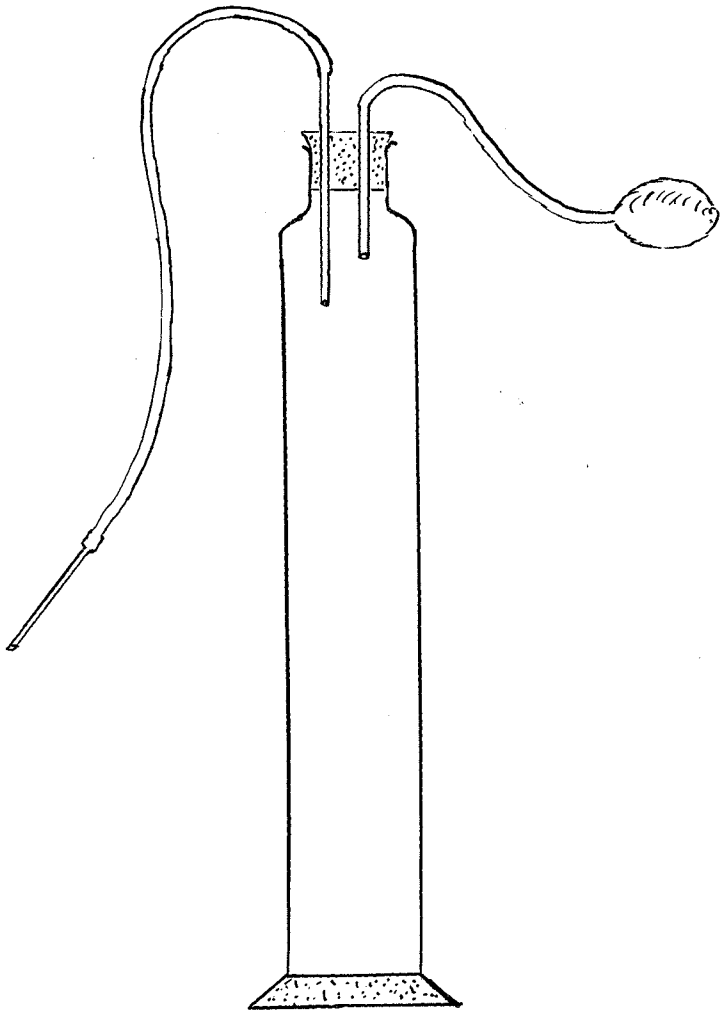
CONCLUSION

These results are not as optimistic for "Merthiolate" as reported by some of the other workers. However, they did seem to indicate that "Merthiolate" in dilution of 1:5000 had a definite bacteriostatic effect, at especially refrigerator and room temperatures, and because of this it was thought advisable to adopt its use. The procedure we adopted, was then to add the "Merthiolate", 1 c.c. of the 2% solution for each 100 c.c. of plasma, to the plasma after it had been drawn off from the blood. (Routine cultures on the plasma were taken prior to the addition of the "Merthiolate").

FURTHER WORK ON BLOOD BANK CONTAINERS AND WITHDRAWAL EQUIPMENT

The other problem, namely finding more suitable equipment, was carried out simultaneously with the above work. It seemed obvious that for a larger plasma recovery a smaller contact surface between the cells

Figure 5

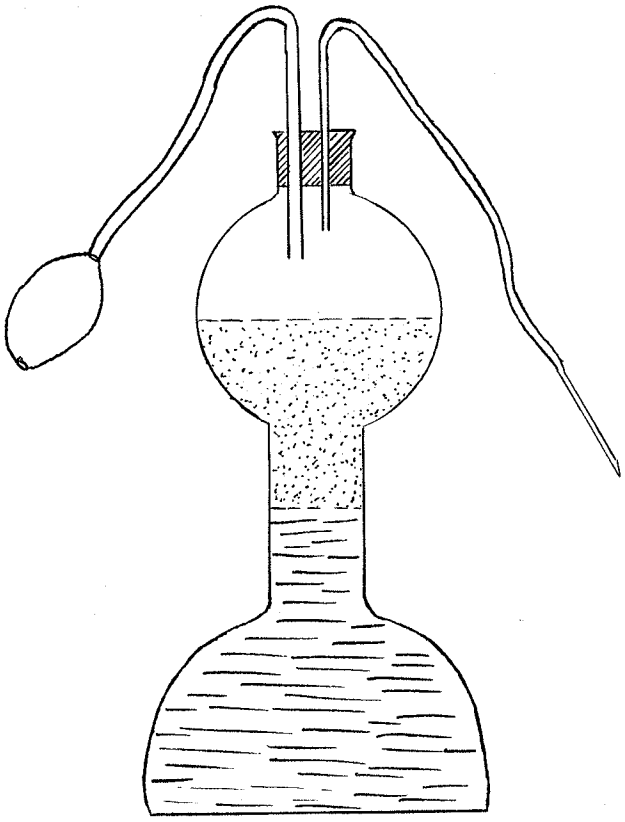


Tall sedimentation cylinder 15.5 inches tall with a diameter of 2 inches.

and the supernatent plasma was wanted. Such a flask would also have to be so constructed that adequate mixing of blood and citrate were possible, else we would get blood clot formation. The first type tried was a tall cylinder measuring 15.5 inches with a diameter of 2 inches. (Fig. 5). The same method of withdrawing blood was used as with the apparatus shown in Fig. 1. By continual shaking during the taking of the blood it was possible to obtain a clot free mixture. Sedimentation of the cellular elements was found to be much slower than in the litre Florence flask; however this was complete in 48 hours. Recovery of plasma from this type of flask was fairly good; approximately 85% of the total possible recovery of plasma was obtained (as compared with 70% in the litre Florence flask). The "shelf-storage" space of such a flask was small. We were, however, unable to obtain any quantity of such flasks with screw tops, so that they were abandoned.

The second type of flask we investigated was the so-called "dumb-bell" sedimentation bottle. (Fig. 6). Several references to this type of bottle were found in the literature. The Blood Transfusion Betterment Association, New York, had had considerable experience with this dumb-bell sedimentation flask, and the following statement about it was obtained by personal communication with Dr. Drew, the medical director of the above association: "The dumb-bell shaped sedimentation bottle in its present form has not proved satisfactory. A plasma yield of 43% is obtained (of the total blood volume)." No reasons for the above statement could be obtained, and it was felt that in spite of Dr. Drew's lack of complete satisfaction with this bottle we would

Figure 6



"Dumb-bell" Sedimentation flask.

like to try them. These flasks were finally obtained, and again we substituted them for the litre Florence flasks (Fig. 1) and used the same technique for withdrawing blood.

CONCLUSIONS

These flasks have been used up to the present date and have proved quite satisfactory. Slightly more care must be taken to insure mixing of blood and citrate, but there is no great tendency to clot formation as there was in the tall cylinder with parallel sides, (Fig.5). The plasma recovery is good; only 10 - 25 c.c. of the total amount of plasma cannot be recovered. Shelf-storage space is fairly small, the base being 4 inches in diameter.

With the above type of apparatus we were still working with a system open to the air, and therefore there still existed that potential danger of contamination that would not be present with a closed system.

EXPERIENCE WITH THE CLOSED VACUUM SYSTEM OF BLOOD WITHDRAWAL

The method next investigated was that of the closed vacuum systems. A complete set was first obtained from the Baxter Laboratories Company.

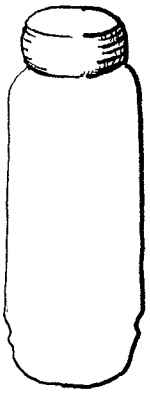
Apparatus - Fig. 7. This consisted of -

(a) A 500 c.c. vacuum centi-vac containing 50 c.c. of sodium citrate, for the collection of blood.

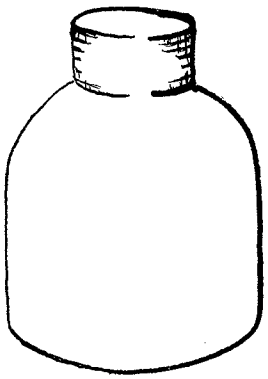
(b) A 500 c.c. plasma-vac, a vacuum flask containing 250 c.c. of 0.9% sodium chloride solution. Into this the plasma was drawn, the resultant mixture giving us dilute plasma.

(c) Transfuso-valve set, for withdrawing blood from a patient or for the removal of plasma.

Figure 7

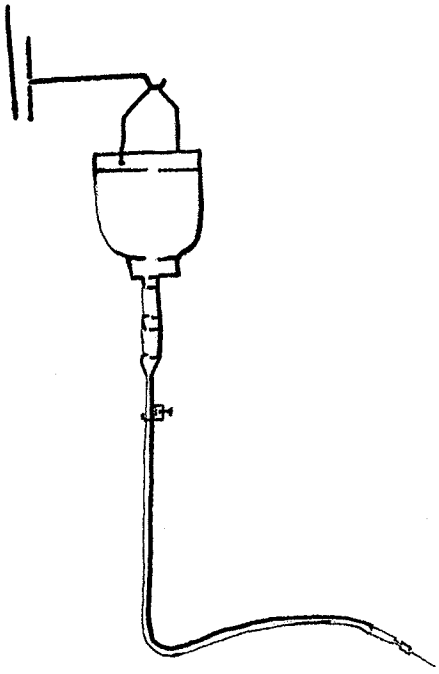


a



b

c



d

Baxter Apparatus
(a) 500 c.c. Centi-vac.
(b) 500 c.c. Plasma-vac .
(c) Transfuso-valve set.
(d) Filterdrip.

(d) A filterdrip, a filter made of stainless steel mesh.

Method of Operation

The needle attached to the valve was inserted through the rubber diaphragm in the collecting centi-vac which contained the sodium citrate. The arm of the patient was then cleaned and the needle at the other end of the valve set was inserted into a suitable vein. The valve was then opened and the blood was drawn into the flask due to the vacuum it contained. When the desired amount was removed the valve was closed, the needle in the diaphragm withdrawn, and still leaving the needle in the patient's vein (the pressure cuff having been released) 5 c.c. were allowed to drop into each of two small bottles, for the cross matching and serological tests.

Advantages

(1) The above described system is a closed system.

(2) It is simple and fairly easy to operate.

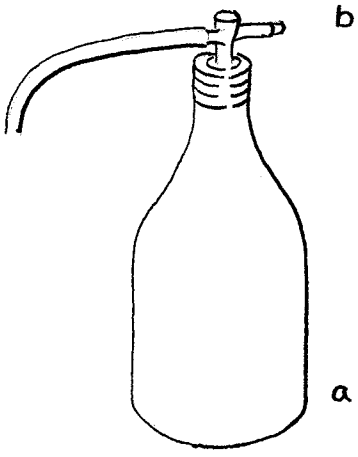
Disadvantages

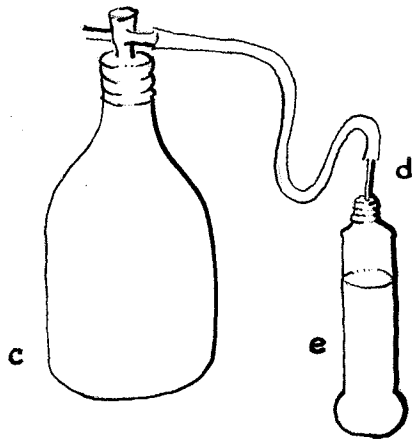
This method of withdrawing and storing blood was decided against mainly because the bottles could not be reserviced for repeated use, and the price of buying new vacuum bottles each time we used a set did not seem to justify the added advantages of a vacuum system.

Following this we obtained the vacuum equipment from the Hospital Liquids Incorporated.

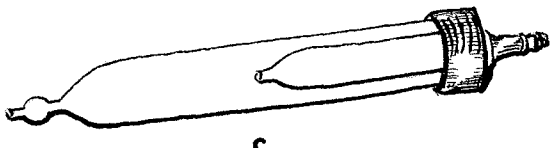
Figure 8

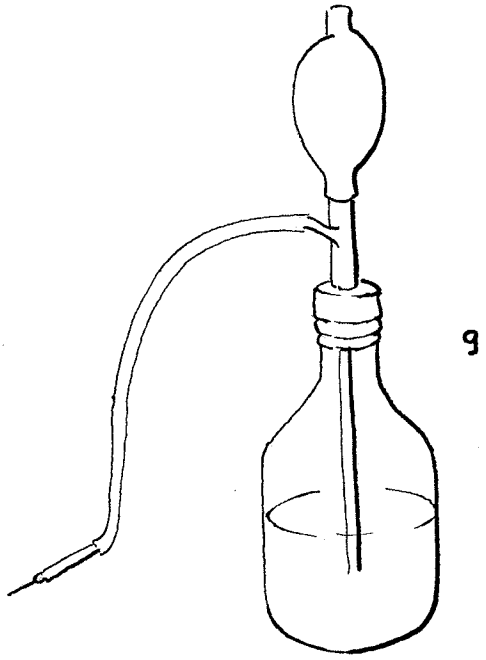
Hospital Liquids Apparatus.

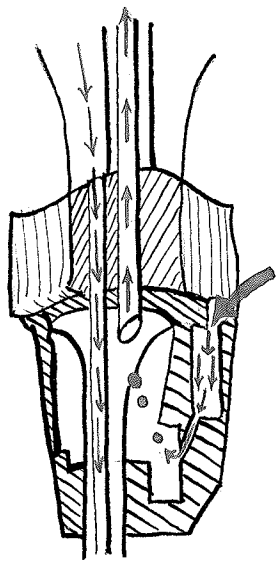




(a) 500 c.c. Hemo-vac for removal of blood.
(b) Valve set.
(c) 500 c.c. empty Hemo-vac flask for storing plasma.
(d) Plasma withdrawal needle.
(e) Sedimentation flask.







(f) Filter.
(g) Emergency set.
(h) Filtrair dispensing cap--green - air intake
--red - outflow of solution.

Apparatus - Fig. 8. This apparatus consisted of the following:

(a) A 500 c.c. haemovac, containing 50 c.c. of 2.5% sodium citrate, used for the withdrawal of blood.

(b) A 750 c.c. vacuum sedimentation container, containing 75 c.c. of 2.5% sodium citrate, used for the removal of blood which is to be sedimented for plasma removal.

(c) Valve set, consisting of a valve, a needle for inserting through the rubber diaphragm, and a piece of rubber tubing with adapter and needle which is inserted into the patient's vein.

(d) Emergency set, consisting of a rubber exhaust bulb with metal valves, attached to a needle.
In case of loss of a vacuum this set is inserted into the flask and sufficient vacuum can be created to continue the withdrawal of blood.

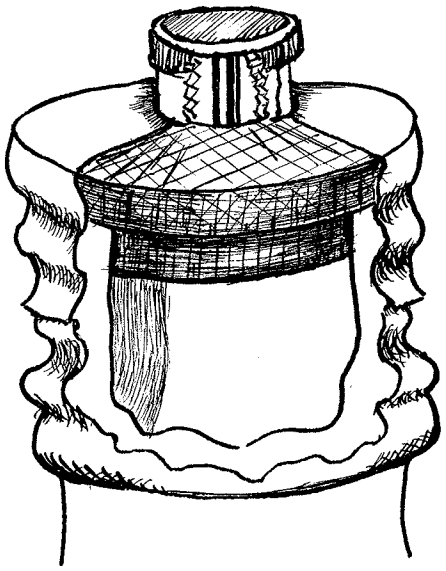
(e) Long plasma withdrawal needle. This is inserted through the rubber diaphragm and down to the plasma-cell junction. The intake of the needle is placed at the side, so that lateral suction currents are created instead of vertical, thus preventing agitation of the surface cellular layer.

(f) Empty 500 c.c. haemovac flask, a vacuum flask into which the plasma may be drawn. For the production of dilute plasma, 250 c.c. of normal saline is added.

(g) Stainless steel filter.

(h) Large 2000 c.c. pooling flask, for the use of pooling plasma.

Figure 9



Hospital Liquid Metal Screw cap showing rubber diaphragm.

(i) Filtrair dispensing caps, contructed from Bakelite, so that the air is filtered through the dispensed fluid before entering into the flask.

Method of Operation

Similar to that described in connection with the vacuum apparatus shown in Fig. 7.

CONCLUSIONS

With this apparatus it was possible to maintain a completely closed system, not only for the collecting of the blood but also for the removal of the plasma. The sedimentation flasks have a small base, thus decreasing the amount of shelf-storage space necessary. The plasma yield from these flasks was slightly less than that obtained when using the dumb-bell sedimentation flasks. We obtained a yield ranging from 40 - 45% of the total blood volume. The most important fact about this type of apparatus was that it was possible to reservice it for repeated use.

OUR MODIFICATION OF THE ABOVE APPARATUS.

All the flasks were originally fitted with a screw thread and a light metal screw cap which could be used only once. (Fig. 9). Brass screw tops to fit these bottles were designed and constructed; these had an inside smooth ring at the top, into which the rubber diaphragm fitted, and a hole in the centre through which the needles were inserted. These were made at the hospital machine shop at a cost of twenty-five cents each. An extra supply of rubber diaphragms (shown in Fig. 9) were obtainable from the Hospital Liquids Incorporated.

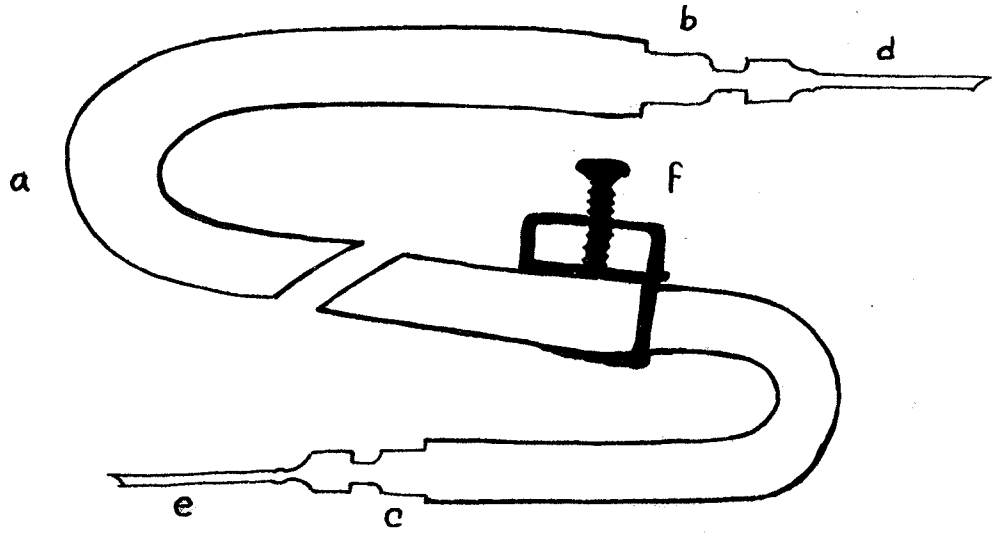
We also found that rubber discs cut out of Latex rubber worked very well. This was made very simply by constructing a punch from a piece of steel piping; a three inch piece of pipe of the required diameter had one end filed down to a cutting edge, with the other end left blunt. The simple principle of condensation of steam, as described by Boland, Craig and Jacobs (1934) and recommended by the British Medical Research Council[7], was used to re-create the vacuum in the bottles.

Method

100 c.c. of 2% sodium citrate freshly made up in a solution of normal 0.9% sodium chloride was placed in the 750 c.c. sedimentation flasks after they had been cleaned. The screw caps containing the rubber diaphragms were screwed on just sufficiently to hold them in place. These flasks were then autoclaved for 30 minutes at 20 pounds steam pressure. The steam pressure in the autoclave was released fairly rapidly, so that the autoclave could be opened while the temperature was still high. As soon as the lid of the autoclave was opened the flasks were removed and the caps on the bottles were screwed down tightly. As the steam inside the flasks condensed a vacuum was created. By this method the occasional flask did not have sufficient vacuum, but on the whole this method was found to work very satisfactorily.

The commercial valve sets were rather expensive, especially since twelve sets were required for our use. We therefore designed and constructed a very simple withdrawal valve set which was found to work very satisfactorily and cost only a few cents.

Figure 10



Simplified Valve Set.
(a) Firm rubber tubing.
(b) Glass adaptor.
(c) Glass adaptor.
(d) Steel needle.
(e) Steel needle.
(f) "Valve" screw clamp.

Description of Simplified Valve Set Fig. 10.

This set consists of two feet of firm rubber tubing. Into either end we placed glass adaptors. The "valve" is a simple screw clamp. We found that a #16 steel needle for inserting through the rubber diaphragm of the vacuum bottle and a #14 or #15 steel needle for insertion into the patient's vein was satisfactory. To insure that no leak into the vacuum occurred an artery forceps was usually clamped on the rubber tubing before inserting the needle through the diaphragm of the vacuum bottle. The rate of flow of the blood is controlled by adjusting this screw clamp. The valve should always be opened slowly, or else the full force of the vacuum may completely collapse the rubber tubing and vein, thus preventing the flow of blood with clot formation.

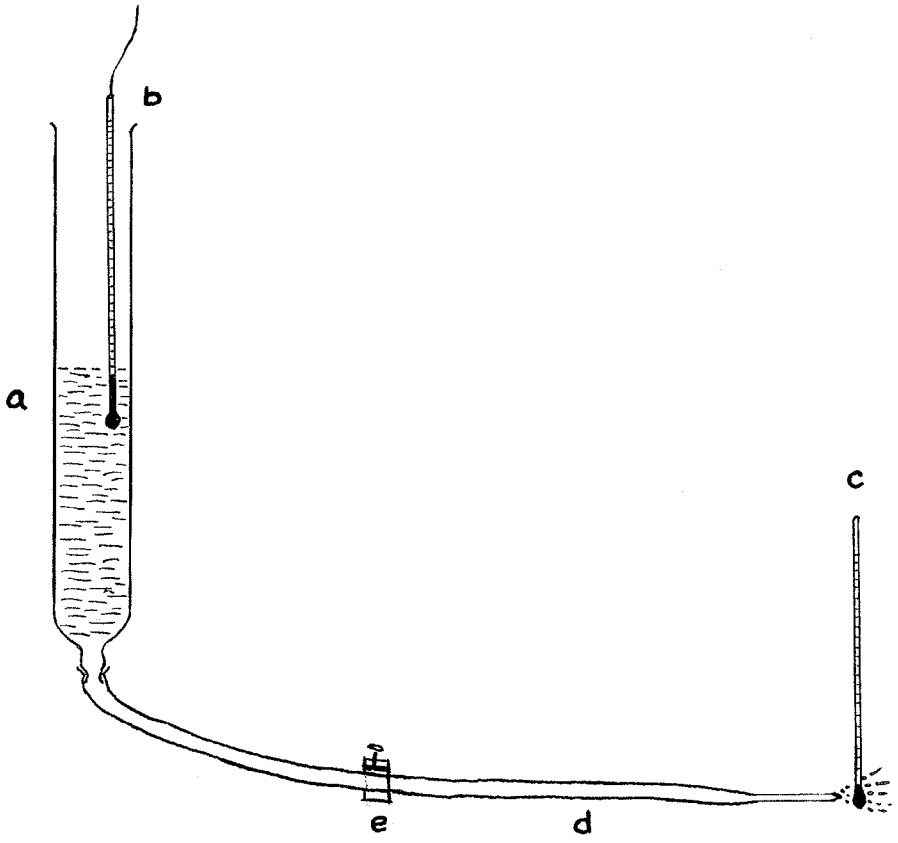
TEMPERATURES AT WHICH BANKED BLOOD SHOULD BE GIVEN AND ITS RELATIONSHIP TO BLOOD TRANSFUSION REACTIONS.

Following an accident on one of the wards, where a flask of blood was badly hemolysed due to excessive heating, the matter of temperature at which intravenous fluids could be given was investigated. Several fatal accidents and severe reactions following the giving of hemolysed blood due to over heating have been reported.(8)

METHOD OF INVESTIGATION

Apparatus (Fig. 11) and Procedure: This consisted of a burette with rubber tubing and an intravenous needle, such as used on the wards at the Winnipeg General Hospital for the giving of intravenous solutions. Three accurate thermometers were also used,

Figure 11



Apparatus for Investigating Temperature Changes.
(a) Glass burette.
(b) Thermometer.
(c) Thermometer.
(d) Rubber tubing with needle.
(e) Screw clamp.

one placed in the burette, one near the needle, and one for recording room temperatures. The solution used was water. The water was allowed to flow from the burette into the tubing and out through the needle. By varying the temperature of the solution originally started with, the rate of flow and the length of tubing, numerous runs were made, each time recording the temperature readings of the three thermometers.

RESULTS & CONCLUSIONS

The following three factors were found to be of importance, namely:

(1) Temperature of the original fluid.

(2) Rate of flow and length of rubber tubing.

(3) Difference in temperature between that of the solution in burette and that of the room temperature.

The results showed that no matter what temperature was started with, either above body temperature or just above freezing point, by the time the solution had reached the needle it tended to approximate room temperature. The slower the rate of flow and the longer the rubber tubing the closer this approximation became. As no attempt was made to keep the burette at a constant temperature (simulating conditions under which solutions are given on the wards) its temperature also tended towards that of the room, although at a slower rate; then it was delivered through the tubing and needle. Calculations were then done in terms of physics of heat, and using the formula(9)

$M_1 (T_1 - T) = M_2 (T - T_2)$ and assuming that an average man received 600 c.c. of fluid which was at 5° C., the body temperature would be reduced by only 0.28° C. With a similar calculation and using fluids at room temperature it gives temperature differences which are negligible. Considerable clinical and experimental data on this subject have been reported by many workers, including De Gowin and Horden,[9] and Page.[10] [11]

CLINICAL EVIDENCE FROM THE WINNIPEG GENERAL HOSPITAL

A. Some forty bloods and plasma transfusions have been given without previous heating. The temperature at which these were given varied from 5° C. to 20° C. No reactions were noted in any of these, nor were there any subjective symptoms reported by the patients.

B. Following the above work the Winnipeg General Hospital in August, 1941, adopted the policy of giving all intravenous solutions at room temperature. As yet there are no accurate figures available, but the incidence of reaction following intravenous therapy is considered to be less than it was for a similar period prior to adopting the "no-heating" policy.

CONCLUSION

From the above evidence we concluded -

(1) That it is a safe procedure to give intravenous fluids at room temperature, and this conclusion is based on clinical and experimental data at this hospital as well as elsewhere.

(2) That the policy of heating blood prior to its being used, unless carefully supervised, is dangerous because of hemolysis which increases the reaction incidence.

PLACENTAL BLOOD BANK

In July, 1941, with the consent and co-operation of the Department of Obstetrics, plans were drawn up for the addition of a placental blood bank to the then existing establishment. It was decided to take blood from all patients, where time permitted and where, in the opinion of the obstetrician, no harm would be done by so doing either to the baby or to the mother.

Placental blood bank bundles were made up, and consisted of the following:

(1) Sterile half-pint milk bottle, covered with a piece of heavy brown paper.

(2) A small bottle containing 20 c.c. of 2% sodium citrate.

(3) Two small screw cap bottles for taking blood for serological tests and for cross matching.

(4) Sterile flats and a 6" x 6" square of Venetian silk.

(5) Cardboard milk bottle top with a ½ inch hole punched out in its centre.

(6) Elastic bands and data card.

PROCEDURE OF BLOOD REMOVAL FROM THE PLACENTA

As soon as possible after the cutting of the cord, the distal ten inches of the cord is to be cleaned with an alcohol sponge and then wiped dry with a dry sterile sponge. A sterile instrument is then used to recut the cord, the end of which is placed through the hole in the centre of the milk bottle cap, which in turn is placed on the top

of the half-pint milk bottle, into which the 20 c.c. of sodium citrate has been poured. The cord is then "milked" or stripped down towards the bottle. When the blood has stopped flowing the bottle cap containing the cord is removed and the bottle is covered with sterile flats and a piece of Venetian silk. Recutting the cord higher up will usually release sufficient blood for 5 c.c. into each of the two small bottles, one containing oxalate for cross matching, the other one for serological tests. After the blood is received by the pathological laboratory two cultures are taken.

RESULTS

Six placental bloods were obtained, with an average yield of 90 c.c. One of the bloods was found to be contaminated, the other five were sterile after three weeks culturing. None of the bloods were used for transfusion purposes, but Wassermann tests, grouping, titers and blood counts were done on these bloods as was followed out in routine for adult blood. Total red blood counts and haemoglobin estimations were above the so-called five million normal for adults. Blood smears showed the presence of some normoblasts. An increased percentage of nucleated red blood cells was found in prematurity.(12) Nothing further has been done with this placental bank, but it is felt that a placental bank could become a worthwhile part of the blood bank establishment at this hospital. In the literature there is still much controversy as to the relative merits or lack of worthwhileness of a placental blood bank. (13) (14)

BLOOD CHANGES ON STORAGE AT 4° - 6° C.

The investigations we did are in no way original, and much work has already been published about these changes. (13) (15) (16) (17) (18) However, the results of these various investigators vary somewhat and we therefore offer our own as another series.

OBJECT OF INVESTIGATION

The object was to determine the successive changes that occur in citrated blood stored at 4° - 6° C. The changes we investigated were the gross and microscopic, which was done by progressive daily red blood cell counts, white blood cell counts with differential counts, platelet and haemoglobin estimations.

METHOD

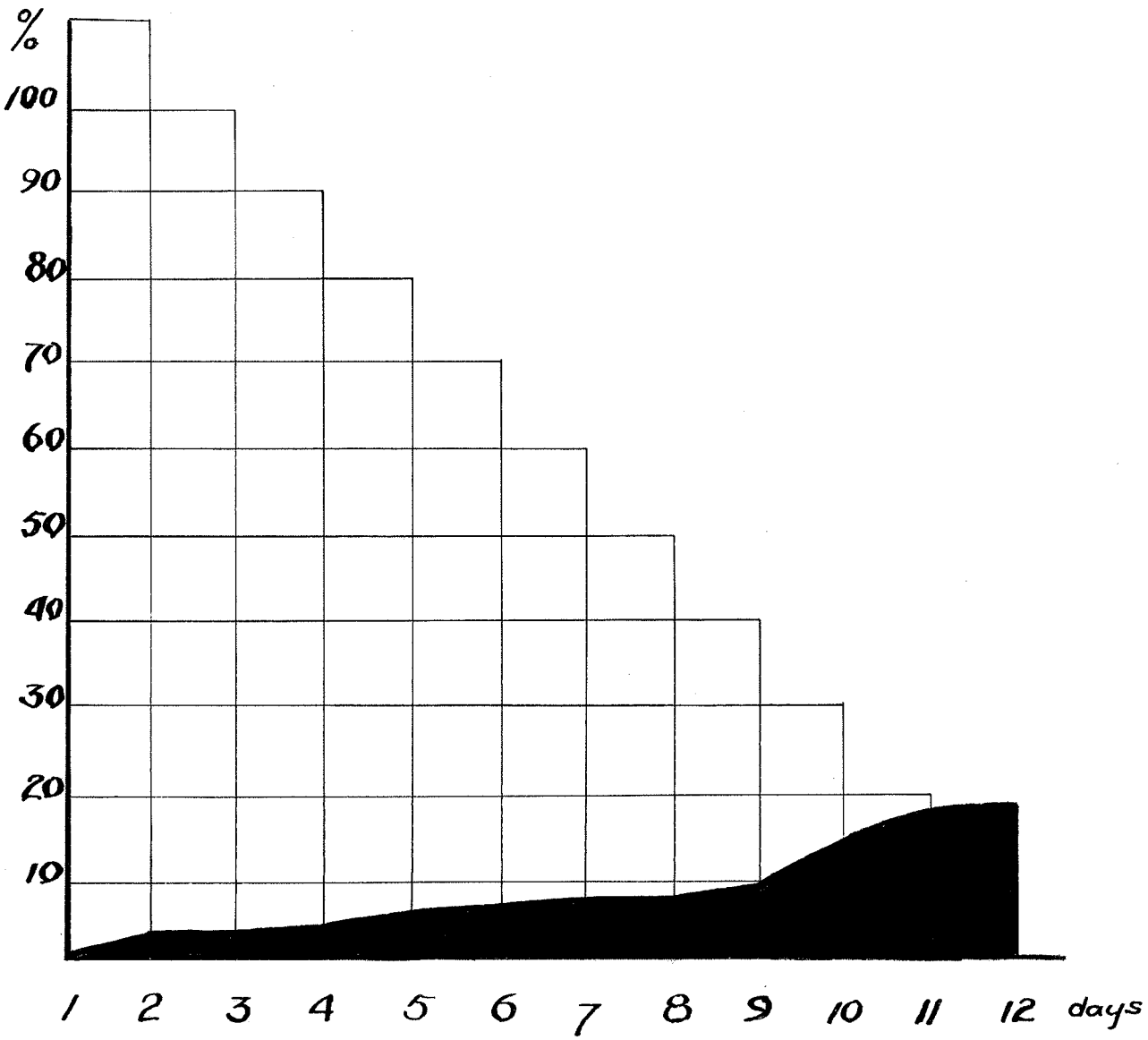
The method used was the "single specimen method", that is, a sample of blood was removed daily from the original flask of blood and the various counts were done on these daily removals. A 2% sodium citrate solution was used as the preservative, and 2 c.c. of this 2% solution was added to each 10 c.c. of blood, giving a final concentration of 0.33% sodium citrate. Haemoglobin estimations were done by the Sahli method and checked by the photo-electric cell method. No correction was made for the dilution factor of the sodium citrate, since it was a constant factor throughout all the counts and estimations. The first counts and estimations, which were doubly checked, were taken as the standard or normal for that specimen, and successive results were then expressed as a percentage of this original "normal."

RESULTS

A. Gross Changes - Hemolysis

At the end of 24 hours sedimentation of the red and white cells is complete. The white blood cells and platelets form a thin, greyish white layer between the red blood cells below and the plasma above. The clarity of the supernatant plasma depends considerably on the time interval that elapses between the donor's last meal and the time of removal of the blood. If this blood is taken within one to two hours after a meal containing fat, the serum is cloudy. We found that on storing the blood hemolysis became evident from the fifth day on. From the fifth to the eighth day hemolysis was very slight, and the plasma in most cases was not grossly colored for more than one centimetre above the white blood cell layer. From the eighth to the fourteenth day hemolysis became increasingly more apparent, so that 80% of the specimens showed fairly gross hemolysis throughout the entire thickness of the plasma layer by the end of the fourteenth day. The individual resistance of the red blood cells to hemolysis varied considerably however. Several specimens showed moderately advanced hemolysis by the end of the fifth day, whereas others showed practically no hemolysis after eighteen to twenty days storage. Since the percentage of haemoglobin throughout the period of investigation remained practically constant (corrections were made for the slight daily variation in haemoglobin), an index of the amount of free haemoglobin (due to actual breakdown of red blood cells) was obtained by taking the percentage difference between the first red

Figure 12



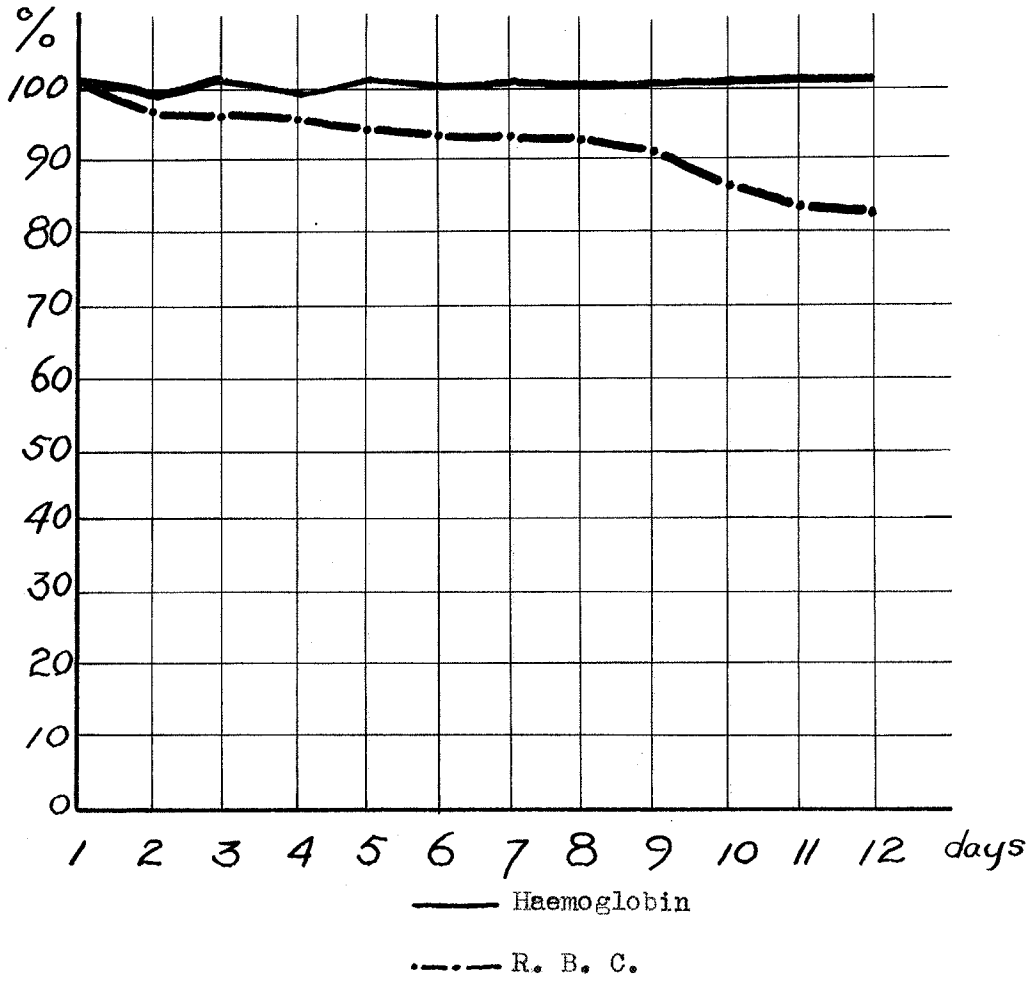
Showing "Units of Hemolysis" occurring during the storage of citrated blood at 4° C. for a twelve day period.

blood cell count and the progressive daily red cell counts. This difference we called the "unit of hemolysis", and this made it possible, by simply doing a red blood cell count on any specimen and comparing it with the original count, to obtain an accurate idea of the amount of free haemoglobin present in any given specimen. The results from 18 cases are shown by the graph in Fig. 12. From this graph it will be seen that until the tenth day the "unit of hemolysis" is below ten, after which period it rapidly becomes doubled. If this unit figure is below ten the amount of gross destruction of red blood cells is comparatively small and therefore the incidence of reaction from free haemoglobin is correspondingly low. When there are twenty "units of hemolysis" present, the blood may still be used, but there is a considerable risk of reaction (not fatal at this level) associated with it.

B. Microscopic Changes

Method of Investigation: Shortly after collecting the sample of blood to be used, two complete and separate red and white blood cell counts were done, as well as two Sahli haemoglobin estimations, (checked by photo-electric machine). The average of these estimations was taken as the so-called standard, equalling 100%. Daily, similarly, estimations were done and expressed as a percentage of the original standard. From daily blood smears differential white cell counts and platelet estimations were made, and the cytology of the cellular elements studied. These studies were done on sixteen different bloods for a

Figure 13



Showing the average daily haemoglobin and red blood cell decrease on storage of citrated blood at 4° C for a period of twelve days.

period of twelve days. Numerous other counts, estimations and studies were carried out as well, some of them on bloods a month old, but since they were not carried through progressively every twenty-four hours they were not included in this series.

RESULTS

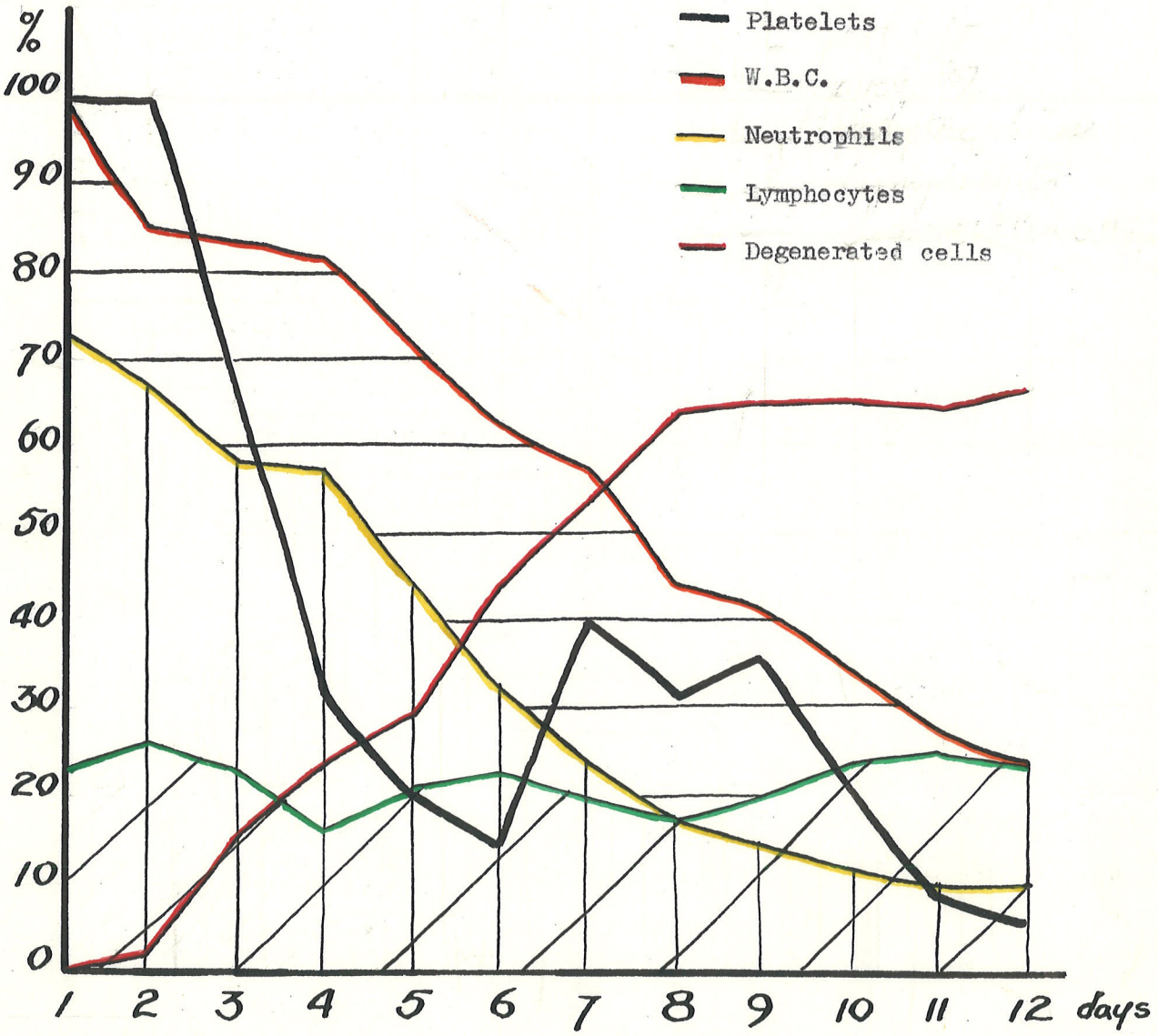
(1) Red Blood Cells and Haemoglobin

The average daily decrease in terms of percentage of the first day's "normal" count is shown in Fig. 13. On the seventh day 12% of the blood showed "clumping" of the cells which could not be broken down by shaking. These clumps were similar to that seen in cross matching with incompatible blood. By the end of the eighth day 30% of the bloods showed clumping. The cytology of the red blood cell at the end of the twelve day period was still considered to be fairly normal. Staining qualities were also good. "Ghost-cell" forms of the red blood cells began to appear from the twelfth to the fourteenth day, and after this their percentage increased fairly rapidly.

(2) White Blood Cells and Differential Counts

Fig. 14 shows the decrease in the total white blood cell count and also the changes in percentages of the polymorphonuclear neutrophils, lymphocytes and degenerate cells. On examination of the blood smears we found evidence of beginning degeneration of white blood cells after forty-eight hours. Changes in such qualities of the cell as morphological characteristics and staining properties were the criteria used for this evidence of degeneration. After four days the polymorphonuclear neutrophils showed marked evidence of degeneration.

Figure 14



Showing the average daily changes of the polymorphonuclear neutrophils, lymphocytes, platelets and degenerated cells occurring during the storage of citrated blood at 4° C for a period of twelve days.

The first cells to show signs of degeneration were the neutrophils, the old ones first and then the younger forms. The lymphocytes, and especially the small lymphocytes, were found to be fairly well preserved at the end of the twelfth day storage period. The eosinophils also maintained their morphology and staining properties for the twelve day period. Lymphocytes were still found in smears of blood twenty days old; they could hardly be called normal but were recognizable.

(3) Blood Platelets

We used a rather less accurate method of estimating the platelet count. They were expressed as platelets per thousand red blood cells, from the blood smears, and then expressed as a percentage of the first or original estimation. The results obtained are shown in Fig. 14. By the sixth day only 15% of the original number of platelets were found present. No explanation can be offered for the rather unexpected rise in count for the seventh and eighth days. By the twelfth day approximately 5% of the original count existed, and these were no longer normal.

SUMMARY OF CHANGES IN BLOOD OCCURRING WHEN STORED AT 6° C.

Fig. 15 summarizes all the above findings of the changes which we found to occur in citrated blood during storage. From this table we see that from the first day on there are recognizable changes in the blood. These changes as detected by the microscope are due to destruction of the cells in the blood. The earliest changes affect the polymorphonuclear neutrophils, which rapidly disappear after the fourth day, at which time half of the neutrophils have undergone

Figure 15

DAYS.

	1	2	3	4	5	6	7	8	9	10	11	12
R. b.c. %.	100	97.2	97.1	96.5	94.8	92.8	92	92	90.5	85.9	82.8	82
"Units of hemolysis"	0	2.8	2.8	3.5	5.5	7.2	8	8	9.5	14.5	17.2	18
Hb %	100	99.5	100.6	99.6	101	100	100.6	100.6	100.6	100.9	100.9	101.5
C. I.	1	1	1	1	1	1	1	1	1	1	1	1
Clumping of R.b.c.	0	0	0	0	0	0	1	2	2	3	3	3
W. b.c. %	100	85.3	83.9	80.9	72.0	62.6	56.8	44.8	40.9	34	27.4	24.1
Polymorph Neutrophil %	72	67.9	59	58.1	44	31.6	24	17.7	15	12	10	10
Lymphocytes %	22.8	26.2	22.9	16.2	20.4	23.1	20	17.8	20	23	25	24
Degenerate W.b.c. %	0	1.5	15.6	23.6	29	43.8	54	63	65	65	64	66
Platelets. %.	100	100	66	32	21	15	40	32	30	20	9	6

Summarizing the changes occurring during the storage of citrated blood at 4° C for a period of twelve days.

degeneration. The progressive fall in the total white blood cell count is almost entirely due to the degeneration of the polymorphonuclear neutrophils. The total leukocyte count is down to half value by the eighth day. The platelets show a rather rapid degeneration, much like the neutrophil cells. The red blood cells are the most resistant to degenerative changes; they begin to hemolyze after the first twenty-four hours, but their destruction is much slower than that of the white blood cells. Sufficient red blood cell destruction has taken place by the tenth to the fourteenth day to show gross hemolysis in the plasma. The above findings are of a similar nature to those found by Kolmer[15], Denstedt[19], MacDonald[7], and Maizels[20].

CONCLUSION

We concluded that from the above findings the maximum optimal storage period for citrate blood was seven days, with an outside limit of ten days. Wiener[21] expresses a similar opinion after a very complete investigation.

REVIEW OF THE USES OF BANKED BLOOD AND PLASMA AT THE WINNIPEG GENERAL HOSPITAL

On November 17th, 1941, thirteen months after the establishing of the bank, the 112th blood had been entered into the blood bank records. This is not however a true figure of the possible "turn-over", since it included all bloods which had been sent to the laboratory, some of which had come from the wards either out-dated, hemolysed or

contaminated before being sent. These were not all a complete loss since we were able to use some for experimental work, as a source of plasma, while much of the remainder was used for making media. The actual number of blood and plasma transfusions received by patients during this period was 52. The blood groups of these 52 were as follows:

(Landsteiner) Group "AB" - 6.7%
" "A" - 42.0%
" "B" - 9.3%
" "O" - 42.0%

The blood transfusions were used for the following types of cases:

Haemorrhage.....5
Ulcerative Colitis.....4
Burn.....2
Diabetic Septicaemia.....2
Post-operative conditions....18
Peritonitis.....1
Severe Anaemia*.....2
Total.....34

The age of the blood varied from twelve hours to eleven days.

. . . . . .

* In one of these cases of severe anaemia a suspension of red blood cells in saline (the cells which were left following the removal of the plasma) was used. The clinical result was satisfactory.

. . . . . .

The plasma transfusions were used as follows:

Nephrosis..... 3

Burns with shock.....11

Hypoproteinemia..... 4

Total.....18

REACTIONS

No reactions were reported, nor were there any untoward effects, objectively or subjectively, that may have been attributed to the use of cold solutions. Several of the patients receiving these non-heated transfusions had high temperatures prior to transfusing.

SEROLOGICAL TESTS

One blood, of the 112 bloods which had serological tests done on them, was reported as giving a "doubtful" Wassermann reaction.

CONTAMINATION OF THE BLOOD AND PLASMA

So far we have had no blood contamination. Several contaminations of plasma have occurred, one of which was due to an obvious break in technique (back-flow of tap water into the flask containing plasma).

PLASMA

The technique of plasma removal has already been described. The plasma removed was kept in two forms, either as the normal concentration of blood plasma or diluted down with equal parts of normal saline and labeled as "dilute plasma."

LENGTH OF PLASMA STORAGE WITH THE CHANGES OCCURRING DURING STORAGE

During the storage of citrated plasma we found slight changes to take place in some of the flasks. After one month, in approximately one-third of the plasmas small whitish flakes began to separate out and collect on the bottom, or else a fine gelatinous mass or "clot" formed. On shaking the flasks containing the flakes, the plasma became cloudy. These precipitates were carefully investigated as to possible bacterial origin. Repeated cultures proved sterile. Staining showed the flakes to be homogeneous structureless masses, so we concluded that this was the fibrin precipitating out of the plasma rather than contamination due to bacteria. This precipitate in plasma is not considered by us to be a contra-indication for use. However, we recommend filtration through a fine mesh steel filter to remove any gross particles.

PLASMA TITERS

The titer of a blood is an expression of the agglutinating strength of its serum (or plasma); it is expressed in terms of a dilution factor. It is the titer of plasma that is the important factor in giving plasma transfusion, rather than its actual group. Most workers now conclude that plasma from any blood group can safely be given to

the patient without previous cross matchings or compatibility tests being done. In considering plasma transfusions group "AB", or Moss group I, is the universal plasma donor, and group "O" (Moss IV) is the least desirable. For this reason we did titers on all group "O" plasmas

Method: Two glass plates, each with nine depressions, are used. With a dropper drawn to a fine point two drops of normal saline are placed in each of the nine depressions. Then into the first depression two drops of the plasma to be tested are added. This is mixed and two drops of this mixture are placed into the second depression. This diluting is carried through until the ninth depression. Into each depression two drops of a fairly heavy suspension of group "A" (Moss II) cells are added and mixed. This gives final dilutions of 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024. The plate is then covered and allowed to stand for 15 minutes. A similar plate is set up for group "B" cells (Moss III). The end point is the least dilution where visible agglutination of cells occurs. Any dilution less than 1:64 is considered to be a true universal donor, and can therefore be safely used. When the titer is higher than a dilution of 1:64 compatibility tests with the patient's cells should be done before using the plasma.

The results of titers done on forty group "O" (Moss IV) were as follows:

True universal donor (0:0 dilution for both "A" and "B")..5%

Below 1:64 dilution (for both "A" and "B")..57%

Between 1:64 and 1:128.....15%

Above 1:128.....23%

The highest titer in this series was 1:1024. There was no relationship between the agglutination titers for "A" and "B" in any one plasma, that is the plasma may be a true universal donor for group "A" cells and yet have an agglutination titer of 1:128 for group "B" cells.

CONCLUSION

From the above figures it is seen that only 60% of the group "O" plasma have a titer low enough to be considered a safe "universal donor", and therefore it is advisable to do titers on group "O" plasma before using, if compatibility tests are not to be done.

DRIED PLASMA

Dried plasma is the final step in the handling of stored blood. The main reasons for drying plasma are:

(a) Safe-guarding against contamination.

(b) Lessening the storage problem

(c) Facilitating shipment of plasma.

It has been shown that plasma can be kept for years, even at room temperature, and still be used without any untoward effects. We do not however feel that it is a safe procedure to store plasma at room temperature, and to store any large amount at 4° C. would necessitate large refrigeration facilities. Lastly, the value of having plasma in such a form that it may be easily conveyed from place to place becomes of added importance because of the present war.

METHODS OF DRYING PLASMA

At the present time all known methods of drying plasma fall under either of two fundamental physical principles:

(a) Simple distillation of fluid plasma under reduced pressure.

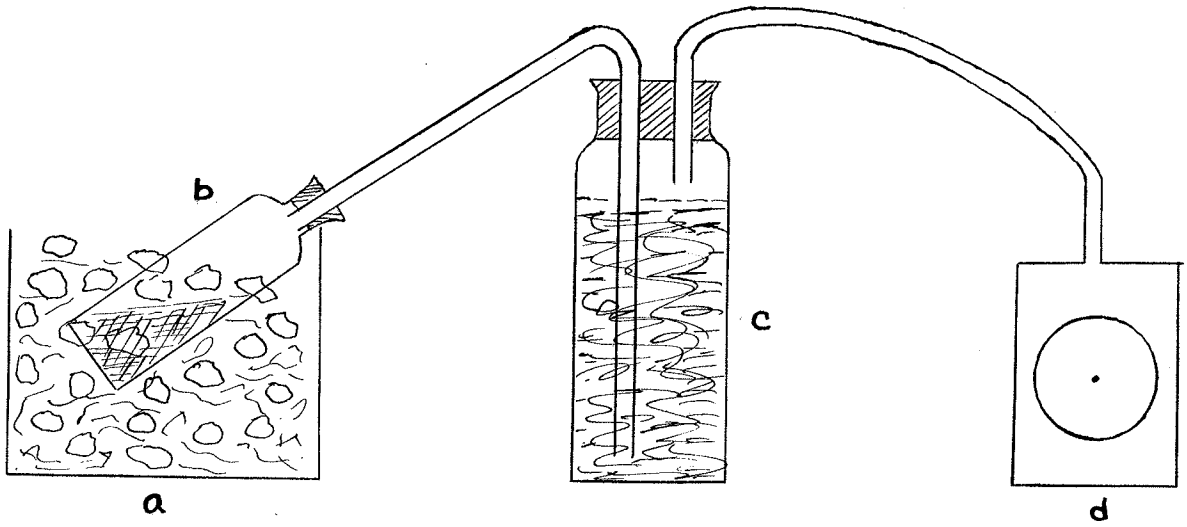
(b) Removal of water by sublimation of water from the frozen plasma.

The desivac process described by Flosdort, Stokes and Mudd[22] and the adtevac process described by Hill and Pfeiffer are the more recent methods for the preparation of a dry plasma from the frozen state. These processes are based on the principles originally described by Shackell[22] in 1909. Various investigators, including Martin[22] in 1896, Edwards, Kay and Davies[22], and Harper and Essex[22] in 1940, have described methods of drying plasma by simple distillation under reduced pressure.

PLASMA DRYING AT THE WINNIPEG GENERAL HOSPITAL

Both methods of plasma drying referred to above were investigated. Because of the complicated and rather expensive equipment required for drying from the frozen state, experimentation was carried out on a miniature scale.

Figure 16



Apparatus for Drying Plasma from the Frozen State.
(a) Freezing bath.
(b) Flask containing plasma.
(c) Absorption chamber containing silica gel.
(d) Cenco Highvac oil pump.

Drying from the Frozen State

The fundamental basis of all methods of drying plasma from the frozen state consists of:

(1) Freezing the plasma, either by creating a low enough vacuum or by reduction of the temperature surrounding the plasma container to freezing levels.

(2) Removal of water vapor by a vacuum pump.

Since no mechanical pump can handle the tremendous volume of vapor (25 c.c. of water on ice produces 236,000 litres of vapor at 0.1 mm. Hg. at 0° C.[23]) some method of condensation or absorption of the vapor prior to its reaching the pump is used.

The method we used is illustrated in Fig. 16, and consisted of the cylinder of plasma surrounded by a freezing bath of ice and salt connected to a flask containing silica gel, which was the vapor absorption chamber. A Cenco Highvac oil pump (loaned from the Cancer Relief and Research Institute) connected to the absorbing chamber was used to create the vacuum. By this method we were able to dry small amounts of plasma. The resulting dried plasma was a satisfactory product, being porous and therefore more rapidly reconstituted into plasma when sterile water was added to it. The cost of building equipment for handling larger amounts of plasma (4 - 6 litres per day) would entail not only a freezing apparatus and a large absorption apparatus, but also the purchasing of an expensive mechanical vacuum pump. For this reason we abandoned the above method in favor of the vacuum distillation process.

PLASMA DRYING BY DISTILLATION IN A VACUUM

General Consideration

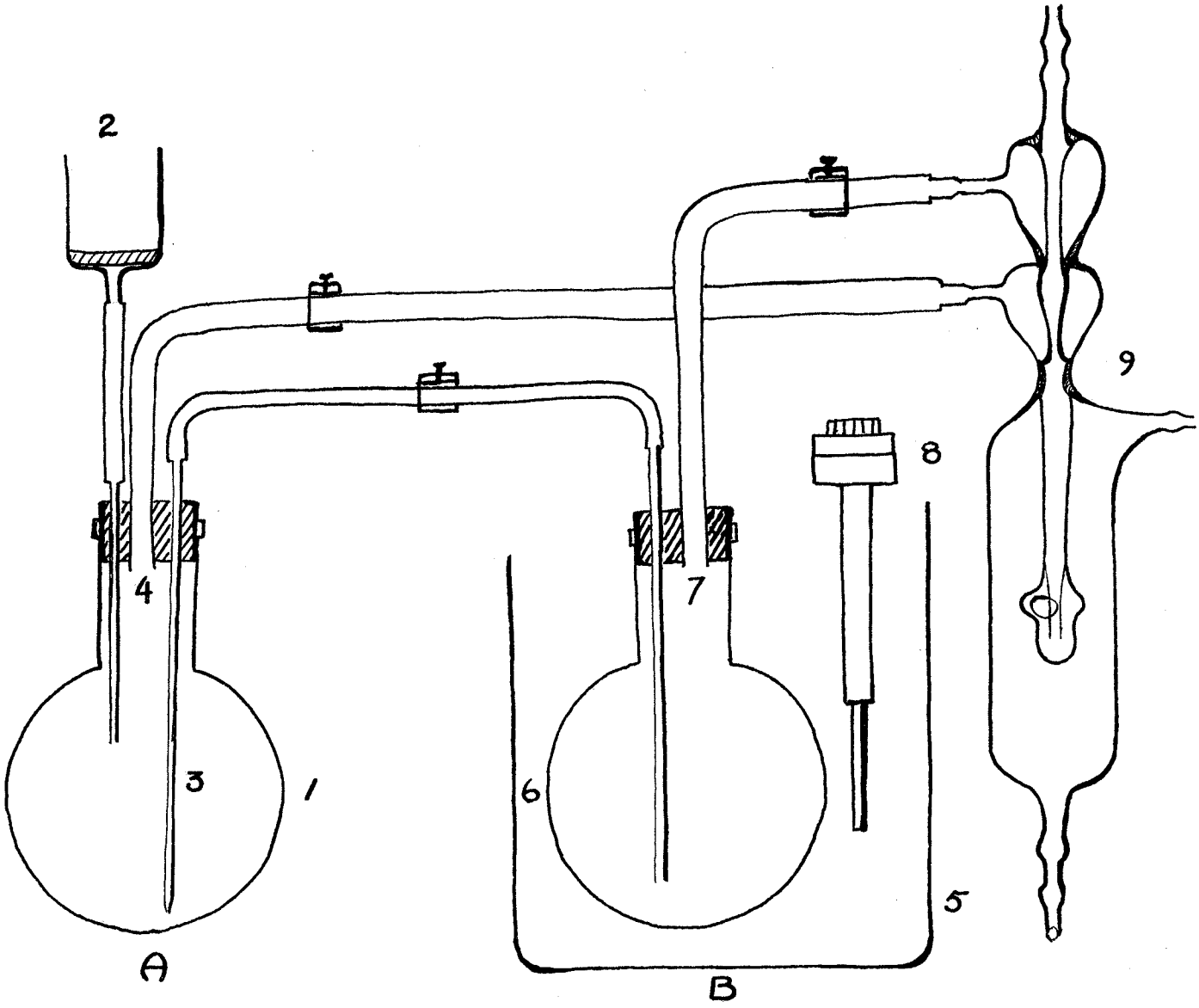
For this type of drying several units of apparatus are necessary - the actual apparatus wherein the drying takes place, a suitable vacuum pump and a constant water bath. A constant temperature water bath is easily maintained by means of a reliable thermostat. The higher the temperature of the bath the more rapid will be the distillation process; however, if this temperature is too high the blood proteins undergo changes. According to Harper and Essex[22] the optimal temperature is from 40° C. to 45° C., although temperatures up to 52° C. may be used.

There are numerous types of vacuum pumps that may be used, such as the water pump, mechanical oil pump and mercury vapor pump. The higher the vacuum the better; however, since simplicity and economy were the object the water pump was decided on. An efficient all glass suction pump with two intakes was used; the pump having two suction intakes eliminated the necessity of two water pumps.

Before actually constructing the drying apparatus the general physical problems of distillation in vacuum were worked on. For mechanical efficiency the following points proved to be of importance.

(a) Increased boiling surface resulted in more rapid distillation. This was achieved by having the plasma run into the distilling chamber at a slow rate.

Figure 17



A Simplified Apparatus for Drying Plasma by Distillation in a Vacuum.

A

(1) 5 litre balloon flask.
(2) Seitz filter.
(3) Withdrawal pipette.
(4) Vacuum intake.

A- De-gassing flask

B

(5) Constant temperature bath.
(6) 5 litre balloon flask.
(7) Vacuum intake.
(8) Thermostat.
(9) All glass aspiration water pump.

B- Distilling flask

(b) Blood plasma contains from 50 to 75 volumes percent of carbon dioxide, and therefore when plasma is boiled a large amount of frothing results. This produced a definite problem. Prevention of frothing by preliminary de-gassing, use of large contact surfaces and re-condensation of any plasma which was drawn off by the vacuum pump, helped to solve this problem.

(c) The larger the vapor withdrawal aperture in the distilling apparatus, the more rapid will be the removal of vapor; also the shorter the distance between the distiller and the suction pump, the more efficiently the vapor will be removed.

By using a completely closed system, sterile plasma, and putting the plasma through Seitz filter prior to its being dried, we eliminated any possible contamination of plasma.

A SIMPLIFIED, EFFICIENT AND ECONOMICAL PLASMA DRYING UNIT

Apparatus

The apparatus used is illustrated in Fig. 17. "A", the de-gassing flask, and "B", the distilling flask, consist of five-litre pyrex balloon flasks. A Seitz filter, plasma removal pipette and $\frac{1}{2}$" diameter vacuum outlet tube completes section "A". The removal pipette is connected to section "B" by means of firm rubber tubing, and the "valve" between these two units is a simple screw clamp. Section "B" is immersed in a constant water bath kept at 42° C. A large glass tank for the constant temperature bath was found to be ideal, since it was then easy to observe the drying process.

In section "B" the vacuum outlet tube is again $\frac{1}{2}$" in diameter. The vacuum pump has already been described. The de-gassing chamber is attached to the second intake of the suction pump, since the vacuum produced by this intake is somewhat less than that produced by the first intake.

Method of Operation

The entire apparatus is set up as illustrated and autoclaved for half an hour at 20 pounds pressure. The sterile plasma is then poured into the Seitz filter and the vacuum to section "A" is started. Because this flask is at room temperature and at a vacuum not so great as that of section "B", the plasma does not undergo vigorous boiling or frothing. In this chamber most of the carbon dioxide is removed and some of the water vapor. After two or three hours of de-gassing, the vacuum to section "B" is begun and by adjusting the screw clamp the plasma is allowed to flow into the second flask at a rate of about 200 - 300 c.c. per hour, which is the approximate rate at which the plasma is dried. On drying the plasma collects on the bottom and sides of the flask. After any given amount of plasma is put through, several additional hours of drying are allowed to ensure thorough removal of water. For removal of the dried plasma from the distilling flask, a long glass spoon or scraper constructed from a piece of glass tubing was found to work very well. The plasma is concentrated to about one-sixteenth of its original weight.

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It may be found advisable to insert a condensing unit between the distilling flask and the suction pump. We found by carefully

watching the process and keeping the rate of plasma inflow just equal to the rate of drying, that a condenser was not essential, and that very little of the plasma was actually drawn over into the pump.

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DISCUSSION OF A BLOOD BANK SET-UP FOR A 500 BED GENERAL HOSPITAL

In our opinion the single most important factor for the successful operation of a blood bank is the blood bank operator. It is this central operator who is responsible, not only for the technical details of handling the blood, but also for the maintenance of the supply from the various departments. This blood bank operator, who should be a trained technician, is under the immediate supervision of the head of the Department of Pathology.

In considering the general problem of a bank for a general hospital, certain facilities and basic equipment are necessary. These would include room space for taking the blood and also bench space for handling the blood. A dust-free cupboard for the removal of the plasma is also advisable. The necessary major equipment includes a refrigerator, the capacity depending on whether or not it is planned to store the plasma as dilute plasma or as dried plasma, an autoclave and an incubator. The incubator and autoclave can quite easily be used in co-operation with some other department, since this equipment is not required for full time operation. The actual amount of blood bank equipment for the removal and storage of the blood cannot be cited exactly; however, the following equipment has proven fairly

satisfactory and would appear to be adequate for the initial setting up of a bank:

(1) 4 dozen vacuum sedimentation flasks.

(2) 4 - 6 dozen haemovac flasks.

(3) 12 of the "simplified" valve sets.

(4) 6 steel plasma withdrawal needles.

(5) Six 2000 c.c. pooling flasks.

(6) 12 dozen small 5 c.c. screw cap bottles.

(7) 6 dozen brass screw caps.

The above is only the major equipment and does not include such things as: depression glass plates for doing titers, grouping serum*, sodium citrate, local sets, Laughlem Reagent for serological tests, record cards and canvas covers in which to autoclave the equipment.

The construction of a plasma drying unit, such as described in the foregoing, greatly reduces the storage and contamination problem and as well makes shipment of surplus supply a simple matter.

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* Grouping serum can easily and cheaply be made in any laboratory. We have used our own product for the past two years and found it quite satisfactory. Method: High titer groups "A" and "B" donors are required, (above a titer of 1:100). Ten c.c. of blood is removed after a 4 hour fasting period, placed in a sterile centrifuge and spun for half an hour. The serum is then pipetted off, and a 2.5% solution of tri-cresol made up in normal saline is added in the ratio of 1 c.c. for each 10 c.c. of serum. We also added one drop of methylene blue to group "A" serum and a drop of safranine to the group "B" serum. Coloring of the serum helped to avoid errors in their use.

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SUMMARY AND CONCLUSIONS

Blood donors supplied from a donors list is not an entirely suitable method of obtaining blood for transfusion.

The open system of collecting in litre Florence flasks is a much less satisfactory method than the closed vacuum system. The Hospital Liquids equipment is the most satisfactory.

"Merthiolate" has a definite bacteriostatic value, but does not give full protection at temperatures above 4° C.

The giving of unheated blood and plasma is a safe procedure.

Placental blood banks are considered to be worthy of further investigation and use.

The optimal maximum storage period of citrated blood is 7 days. By this time the polymorphonuclear neutrophil cells have disappeared, platelets have greatly decreased, but there is as yet practically no change in the red blood cells and hemolysis has not yet begun.

Fibrin precipitation occurs in citrated plasma after storage for 30 days. This is not however a contraindication for its use providing it is filtered.

Plasma titers lower than 1:64 are safe to use without previous compatibility tests. Only 60% of group "O" fall into this safe class.

A simplified inexpensive method of drying plasma by distillation in a vacuum is described.

A single responsible blood bank operator is the one most important factor in the successful operation and maintenance of a blood bank.

Appreciation

I am indebted to Dr. H. Coppinger for having made possible the establishment of the blood bank.

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