

HUMAN BLOOD: Life Saver 1 in World War II

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THE use of human blood in the practice of medicine dates back many centuries. Successful blood transfusions probably were first performed in the middle of the seventeenth century. In some instances the blood of animals was employed, but such a procedure frequently resulted in serious reactions; transfusions of human blood were more successful. Even these were subject to two serious drawbacks; namely, the technical difficulties related to the actual transfer of blood from one individual to another, and reactions resulting from the use of the wrong type of human blood.

Foremost among the technical difficulties of transfusions was that caused by the inherent tendency of the blood to clot outside of the body. To remedy this handicap, removal of the fibrin from blood by mechanical defibrination methods was first employed; it was nearly a century later, in 1914, that sodium citrate was introduced by Hustin as an anti-coagulant for blood. The epochal experiments of Landsteiner in 1900 on agglutinating substances in human blood led to its separation into four types and removed the other serious drawback to transfusion. Now it became a comparatively simple laboratory procedure to determine the proper blood donor for a given patient.

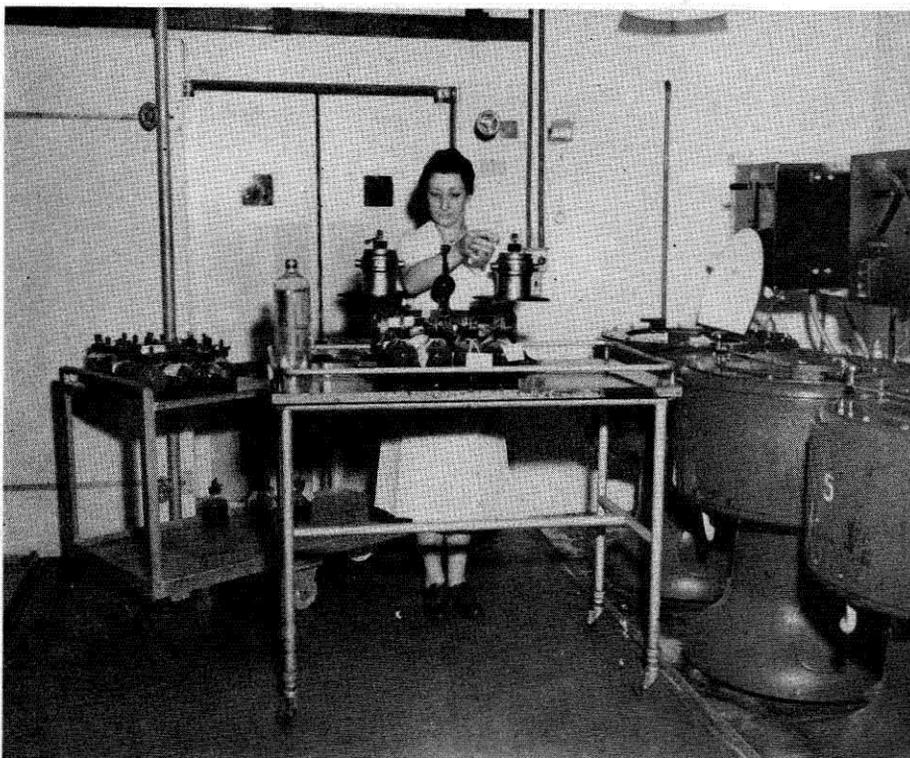
BLOOD TRANSFUSIONS UNDER WAR CONDITIONS

The complicating factors related to the use of human blood in military medicine were strikingly illustrated by its almost complete absence during the Civil and Spanish American Wars. It has been pointed out that the Surgeon General's History of the Civil War records but two blood transfusions; even 35 years later, during the war with Spain, blood transfusions were apparently not used in military medical practice. During World War I there were extensive developments in blood transfusion tech-

niques. The use of blood directly was almost universal since storage of the material in blood banks had not been developed.

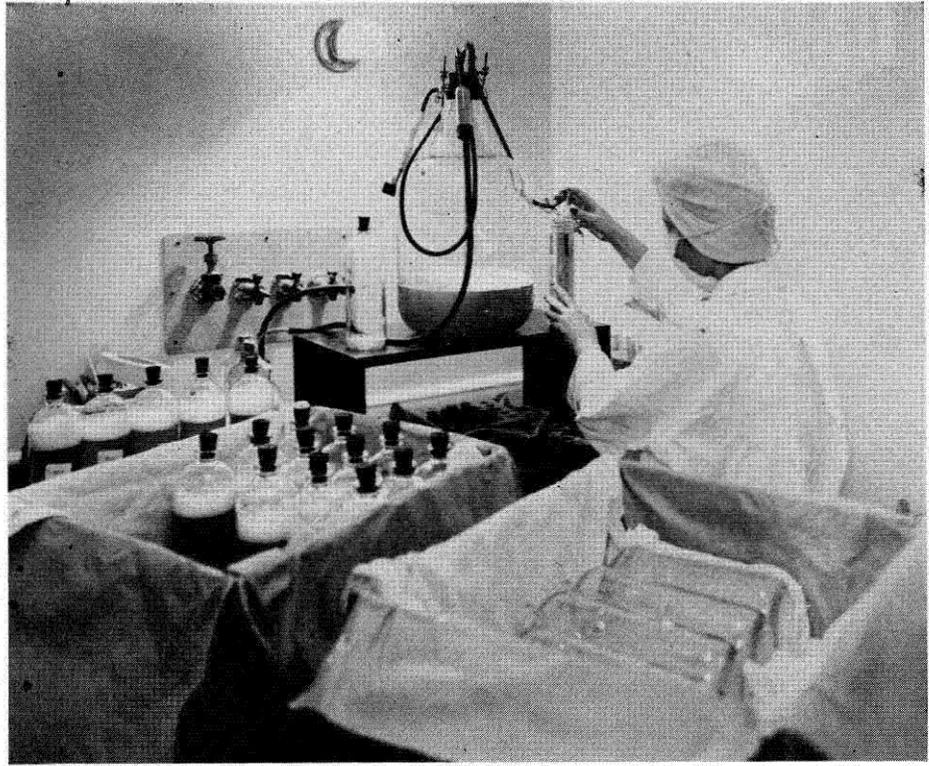
In recent years the maintenance of stores of sterile, typed bloods has proved of tremendous value in civilian medical practice. However, such blood preparations deteriorate rapidly and are good for only a few weeks at best, even when kept under ideal cold storage conditions. This instability of stored blood is a great handicap to military medicine, so numerous products have been prepared from human blood to meet the emergencies of war.

The composition of blood may be roughly classified under two divisions; namely, (1) the plasma or fluid portion, and (2) the cellular constituents, including red corpuscles, white corpuscles, and blood platelets. The prevention and treatment of shock resulting from severe wounds and burns under combat conditions are, of course, now a major problem in military medicine. It has been found that the plasma portion of the blood is just as effective as whole blood in the prevention and treatment of shock, except in those cases where a tremendous loss of blood occurs and the red blood cell concentration falls low enough to produce a fatal anemia with anoxia. Since fatal shock almost always occurs *before* this level is reached, the primary problem in most cases is one of preventing and treating shock. Plasma and similar human blood products are excellent for this purpose. Military experience emphasizes the fact that there is little loss of red cells in severe burns; rather there may be a severe loss of plasma from the circulating blood with an increase in hemoglobin concentration. Consequently, plasma rather than whole blood appears to be indicated for this condition.



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FIG. 1: Balancing the load in cups before centrifuging the blood to separate the plasma from cellular constituents.



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FIG. 2: Dispensing pooled plasma into final glass containers.

The use of plasma in place of blood for the treatment of shock has substantially removed one of the major technical difficulties associated with transfusion, namely, the necessity of typing bloods in order to select proper material, since blood type incompatibilities are largely associated with the cellular constituents. In practice, a considerable number of plasmas from bleedings of various types are pooled before processing, diluting the substances present which might in some cases give untoward reactions. Consequently, this pooled material may be freely used in the field without testing for blood type.

Plasma prepared from citrated blood and serum from clotted blood are standard preparations that have been widely used in the present world conflict. While these products are far more stable than whole blood, they are too labile to be ideal for medical practice in the military field. As a result, two additional products have been prepared from human plasma: dried plasma and albumin.

BLOOD COLLECTION

Blood collections by the American Red Cross at various bleeding centers throughout the United States are made in sterile assemblies previously prepared at the processing laboratory. A typical bleeding unit consists of a bottle containing a small amount of sodium citrate solution, stoppered with a special rubber closure. This assembly includes a two-holed rubber stopper fitted with two small stainless steel cylinders with rubber tubing attachments. A small cotton filter is attached to the end of one of the rubber tubes, while the other one is fitted with a bleeding needle shielded with a small test tube to help maintain sterility during transportation and temporary storage, before use at the bleeding center. Each complete bleeding assembly is sterilized with steam in a pressure autoclave. These sterile collecting assemblies, prepared at various processing laboratories throughout the country, are shipped in special refrigeration boxes to the Red Cross bleeding centers. In practice, it is, of course, not necessary to keep the sodium citrate solution

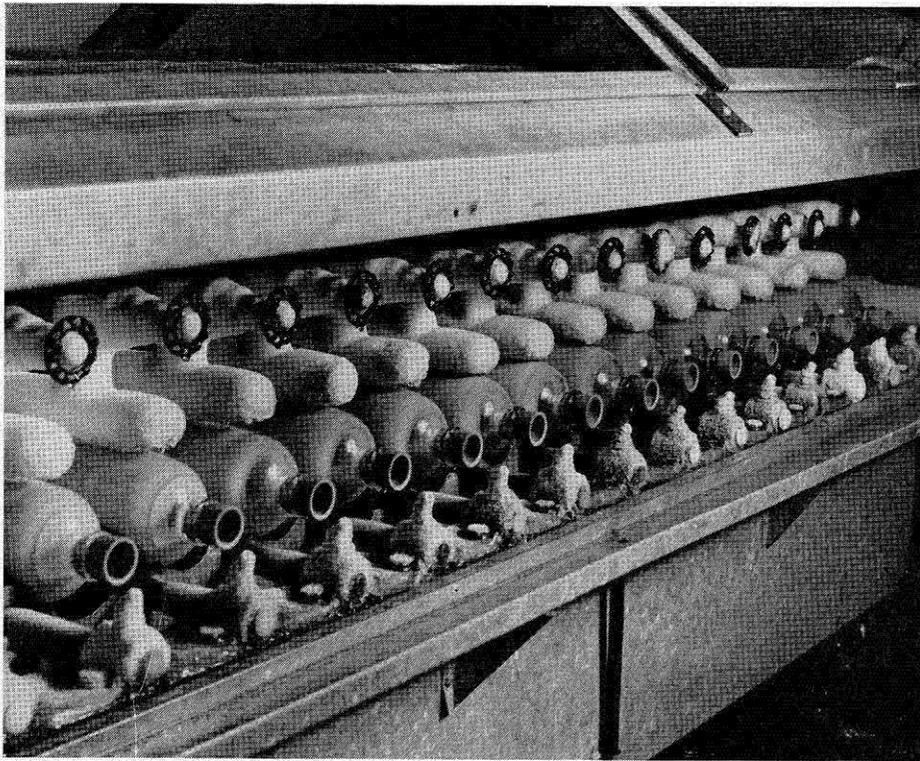
cold, and the refrigeration boxes are iced only during their return trip from the bleeding center to the processing laboratory.

After the desired amount of blood has been obtained from the donor, small spring clamps are placed over the two rubber tubes, the longer of which is then cut off close to the rubber stopper. The blood remaining in this tube is subsequently drained into the test tube which has served as a shield for the bleeding needle. The test tube sample of blood is stoppered and shipped back to the processing laboratories with the bleeding; it serves for necessary serological tests. The bleeding bottle proper is placed in a standard refrigerated box.

These refrigerated boxes are expressed immediately to the processing laboratory and the bleedings stored in a cold room pending completion of serological tests. Samples for these tests are checked, recorded, and delivered to biological control departments. Any bleedings which prove to be unsatisfactory by such tests are removed so that they cannot cause contamination in the pools.

SEPARATION OF PLASMA

After the bleedings have satisfactorily passed the serological tests, they are ready for the separation of plasma from the cellular constituents. Although the cells of citrated blood will settle quite well on standing, it is common practice to centrifuge the material in order to speed up the rate of settling. This treatment also increases the plasma yield. Cup-type centrifuges are used and these are refrigerated to keep the blood cold during the process. Since they are operated at maximum speed and are run almost continuously, it is necessary to balance the load in the cups very carefully (see Fig. 1). A centrifuging period of about an hour gives an excellent packing of the cells. Any tendency for the red blood cells to hemolyze and yield red plasma is minimized by refrigeration during the shipment of the blood, as well as during subsequent storage and separation of the plasma. After the bottles have been centrifuged, they are



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FIG. 3: Equipment for rapid freezing or "shelling" of liquid plasma. Refrigerant flows over rotating bottles.

very carefully removed in order not to disturb the cells and are placed in the cold room to await pooling.

PLASMA POOLING

To eliminate traces of blood type incompatibilities, plasma representing from 50 to 55 bleedings is mixed in a large pooling bottle. This process allows the plasma to be administered readily and safely to a person of any blood type without undue reaction, which is very important in emergency battlefield treatment. The pooling operations and the filling of final containers are carried out in special air-conditioned rooms (see *Fig. 2*). The air entering these rooms is not only filtered through the best available equipment, but is treated with ultra-violet light in order to further reduce the bacterial count. The flow of filtered air is maintained at a sufficient rate to represent a complete change every three minutes. Moreover, the rooms are frequently flooded with steam between pooling operations. Strict aseptic precautions are observed throughout all operations, and only specially trained technicians dressed as for surgery are allowed in these rooms.

In the pooling process the supernatant plasma is drawn off by vacuum through a closed system into a large pooling bottle with the aid of a special-design aspirating spoon. The pooling bottle is then shaken vigorously to insure thorough mixing of the plasma. Two samples for sterility testing are taken immediately from each bottle and are sent to biological control departments. A bacterial preservative is then added to the pooled plasma, and a third sample of 50 cc is taken in one of the standard final containers. This is then carried through the entire plasma processing procedure along with the regular material, including freezing and drying, and is used as a check on the sterility of the plasma pool represented.

FREEZING OF LIQUID PLASMA

The pooled plasma is now dispensed into final glass containers—tall, cylindrical bottles with an overall

capacity of about 750 cc. Approximately 550 cc of the plasma (equivalent to two Red Cross bleedings) are added to each of these bottles. The plasma is now ready for rapid freezing or "shelling." This is accomplished either by rotating the bottles in a low temperature bath containing a freezing mixture or by flowing the chilled refrigerant over the bottles as they are rotated (see *Fig. 3*). Ideally, the material should be frozen as a uniform shell on the sides of the bottle with a hollow core clear through the center. In practice it is found that the plasma tends to freeze in the neck of the bottle, so that local application of heat from a small steam coil is frequently resorted to in order to prevent this freezing. It may also be necessary to warm the bottom of the container while it is rotating to prevent too heavy a deposit of frozen plasma there. If the frozen plasma is not properly proportioned in the bottle during shelling, irregularities in the drying cycle occur, slowing down the process and occasionally injuring some of the material.

The proper quick freezing or "shelling" of the plasma called for the development of equipment, since no stock machinery was available. Various modifications of the types of shelling equipment have been developed throughout the industry; the two described above represent those most commonly used. A third ingenious device consists basically of rotating spindles on which the bottles of plasma are mounted. The speed of rotation of these spindles is such as to cause the plasma to form a rather uniform layer against the inside of the bottle, due to centrifugal action. These bottles maintained at proper rotation are then refrigerated and subjected to a blast of very cold air which causes the plasma to freeze quickly. A hollow core in the center of the bottle naturally results from the above procedure, although the force of gravity causes the layer of frozen plasma to be thicker at the bottom of the bottle than at the top.

It is essential in all these "shelling" procedures that the necks of the bottles be kept completely clear of frozen plasma in order to facilitate subsequent rapid drying in vacuo. Considerable experimentation and adjustment of



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FIG. 4: Rubber stoppering of dried plasma bottles as they are removed from the desiccation trays.

the equipment are necessary to produce a fully satisfactory frozen plasma. Following freezing of the material, the bottles are stored in a cold room at -20 degrees Centigrade.

DRYING OF FROZEN PLASMA

Several types of equipment are commonly used for drying the frozen plasma. Since the product must be kept substantially free from bacterial contamination throughout, the equipment and procedure requirements are rigorous. Two sources of vacuum are commonly employed; namely, (1) mechanical pumps, and (2) multiple stage steam ejectors. When the high vacuum mechanical pumps are used, it is necessary to have an effective condenser for cooling the water as it evaporates from the frozen plasma. These condensers must be maintained at a low temperature (-40 to -60 degrees Centigrade) in order to collect the greater part of the water vapor and protect the pump from overload.

In the case of the steam ejector type of vacuum equipment, the capacity of the system is large enough to handle, without aid of a refrigerated condensing unit, the water vapor given off. A multiple stage ejector producing a vacuum of around 100 microns under no load is usually employed. Jet condensers with barometric legs and a large cooling tower complete the major pieces of equipment required for the steam ejector vacuum assembly. In practice, a vapor pressure range of 500 microns down to about 100 microns is employed during the drying cycle.

The desiccating chambers for this equipment essentially consist of vacuum cabinets of various sizes and designs. Multiple small units may be used. These are manifolded to the main vacuum line—or one or two very large vacuum chambers may be used. In either case it is necessary to gradually supply carefully controlled heat to all bottles of frozen plasma. This may be accomplished either with the aid of electrically heated plates or with hollow shelves whose temperature is regulated by the circulation of thermostatically controlled hot water.

The problems of heat flow under these high vacuum conditions are rather extensive, and they require a great deal of engineering design and subsequent alterations on a trial and error basis.

An alternative method of drying the frozen plasma consists in mounting the bottles on individual vacuum lines. Such an assembly has been termed the "Christmas tree," with the main vacuum line representing the trunk, the large feeder lines corresponding to the branches of the tree, and lines for individual bottles corresponding to the tips of the tree branches. This type of device can be moved from one room to another, since higher temperatures are required at the latter stages of drying. A flow of heat through the frozen plasma results from warm air around the outside of each of the bottles. This system has one serious drawback—the breaking of one container will temporarily destroy the vacuum of the entire system, not only slowing up the process but possibly resulting in a denaturation of some of the partially dried plasma.

At the start of the drying run, the temperature of the plasma is well below freezing (-20 to -30 degrees Centigrade). It is gradually increased as the moisture content is lowered, and in the final drying stages a temperature as high as 50 degrees Centigrade may be employed in order to yield material containing less than one per cent water. It is essential to keep the final water content very low in order to have maximum stability in the dried plasma. Drying from the frozen state minimizes the tendency to denaturation of the sensitive proteins present, and the properly dried final material has such a stability that it may be kept without refrigeration for years and still be fully satisfactory for clinical use.

PACKAGING OF DRIED PLASMA

After drying is completed, the containers are removed from the vacuum chamber with full aseptic precautions. (See Fig. 4). The containers are closed with sterile rubber stoppers by a crew of specially trained workers, also



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FIG. 5: A marine of the Fourth Division on Saipan receiving blood plasma—almost as soon as he was wounded. The process takes from eight to 15 minutes and prepares the wounded man for removal to the operating hospital.

dressed as for surgery. The stoppered bottles are then taken to a hand-operated special vacuum machine which removes the stopper momentarily, evacuates most of the air from the container, and replaces the stopper. The hood of the sterile stopper is immediately turned down to protect the edge of the bottle, and next the outside of the stopper and the neck of the bottle are given a protective adhesive coating to assist in maintaining high vacuum until the plasma is used.

Representative samples of the final packaged containers are then subjected to rigorous tests to insure a sterile product. Safety tests in laboratory animals are also made. The dried plasma, now ready for final packaging operations, is placed in a can which is evacuated, flooded with nitrogen and hermetically sealed. It is believed that the presence of an inert gas like nitrogen will materially prolong the life of the rubber stopper and will also tend to minimize deterioration of the dried plasma if a possible leak in the vacuum of the bottles occurs. A bottle of sterile diluting fluid (distilled water containing a small amount of citric acid), identical in size with the plasma container, is supplied along with the bottle of dried plasma. These two with the injection equipment constitute the complete outfit. Because of the porous consistency of the plasma and the fact that it has not been appreciably denatured during drying, solution is very rapid when the diluting fluid is added to the evacuated plasma bottle. It is possible, in expert hands, to bring this about in less than a minute, and thus an effective preparation is ready for almost immediate administration in the field.

These plasma kits are placed in a waterproofed bag before sealing, and are packaged twelve to a carton. The waterproofed bag enables the cartons to float in case an emergency necessitates dumping the plasma before the ship reaches shore. The cartons thus packed are then bound with tin strips and are ready for shipment.

OTHER HUMAN BLOOD PRODUCTS

Mention should be made of some of the other human blood products that have been developed in connection with the Red Cross blood program. The albumin fraction of the plasma, constituting about 60 per cent of the total protein present, has been found to be an excellent substitute for blood in the prevention or treatment of shock, and is now being used by the armed forces. It may be separated in a highly purified form from human plasma by chemical fractionation in the cold (about -5 degrees Centigrade) with the aid of alcohol. The product so obtained is dried in trays from the frozen state to remove the residual alcohol. It is then dissolved in water containing a small amount of *NaCl* and a preservative, and is ready for final filtration and filling. A sufficient amount of dissolving fluid is used to yield a solution with a final concentration of 25 per cent albumin. Since it is not practical to carry on the process under completely sterile conditions, the final solution is rendered sterile by being passed through one of the standard bacteriological filters. It is necessary, of course, to adjust the *pH* of the final product to about neutrality in order to insure stability and harmlessness on injection. The 25 per cent albumin solution is dispensed in 100 cc amounts in special hard glass containers. It has a pleasing yellow-green color, is crystal clear, and is remarkably stable even at tropical temperatures. This stability allows it to be freely used in military operations where space is at a premium and refrigeration may not be available. The 100 cc vial of albumin is packaged in a tin can along with the required administration equipment. Since it is dispensed as a liquid, no immediate diluting fluid is needed; so the space required is very small. One vial of albumin solution is approximately equal to two human bleedings in terms of shock-preventing properties.

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be conforming with W.P.B. policy as outlined under the "Spot Authorization Plan." Such a company would have available excess capacity *not required in war production*. They would have *labor available*, the use of which would not interfere with labor requirements for war production in that area. They would furthermore be producing a scarce and essential civilian item—a fact that the O.P.A. has recognized for over a year, as proved by its distribution of stoves under a rationing program. Thus when Amendment 1 to MPR 64 was issued on August 11, 1944, the O.P.A. must have been fully aware that this was a pricing policy to be applied in an industry where an approved reconversion plan was in operation.

PROFITS

This must then be considered as a reconversion pricing policy and examined in the light of its probable effect in the stove industry. This industry has been largely engaged in war production. As war contracts terminate, each company must consider its re-entry into the stove business in the light of this price regulation. Unless established ceilings are high enough to provide a profit, the best the manufacturer can hope for is a break-even operation and that only if he can meet the prices of his lowest competitor. Two probabilities suggest themselves.

A large percentage of the average manufacturer's output is in "low cost" production of low-profit, large-volume items. These are in contrast to slower-selling, higher-priced, larger-profit models. Under stringent price control, the low-cost production would be discontinued and, as a severe consumer shortage exists, the higher priced models would be sold exclusively. The entire O.P.A. policy is thus circumvented as overall cost to the consumer is increased as a result of the attempt to control profits.

Not all manufacturers will be able to realize a profit even on their most profitable models. Such companies might, in view of this no-profit order, decide to enter an entirely new field. This would, in the first place, retard the manufacturer's reconversion, as presumably retooling, altered plant layout, engineering design, sales policies, and a host of incidental problems would prevent his speedy resumption of full-scale operations. Such a delay is generally considered to be the most likely cause of a serious unemployment problem following the war. In the second place, the loss of any considerable portion of the stove manufacturing capacity would prolong the present shortage, make normal competitive pricing more difficult to attain, and finally threaten a definite hardship to the civilian population, as cooking and heating equipment is essential to health and comfort.

Since a manufacturer faced with this specific problem would surely choose one of these two courses of action in preference to continued operations at a loss or at the best on a break-even basis, it is difficult to see how either O.P.A. or overall government policies can be realized through such pricing regulations. It is to be hoped that once again the experience gained in the operations of reconversion pricing in the stove industry will guide the way to a more effective and workable control to insure high levels of production and employment.

Human Blood

(Continued from Page 8)

Other products which have been obtained from human plasma and show real value include: (1) immune globulin used to control epidemics of measles and scarlet fever, and (2) thrombin used with foams prepared from human fibrin. These foams or sponge-like preparations,

together with thrombin solutions, are of special value in brain surgery for the control of bleeding and may be left in place following the operation, since they are ultimately absorbed.

One of the most recent and important developments is found in the special anticoagulant solutions for preservation of whole blood. Solutions of this type have been prepared which now make it possible to send blood transfusions directly from this country to all the battle areas (thanks to modern air transportation). It is necessary, of course, to carefully type these whole blood preparations so that the patient is sure to receive the right kind of material. These whole blood units are of great value in the treatment of those casualties where extremely heavy losses of blood have occurred.

FIELD RESULTS

Reports from the South Pacific and other fighting fronts describe the use of plasma and albumin on the battlefields. Casualties are given transfusions at aid stations a few hundred yards from the firing line, some 10 to 30 minutes after being wounded (see Fig. 5). The process, taking from eight to 15 minutes, prepares the wounded men for transportation by litter back to the operating hospitals, by restoring the bulk and balance in the blood stream and counteracting the effects of shock. Navy Medical Corps men say that the tins of plasma are as easily handled and transported as cans of food, since they are protected from weather and breakage and are not affected by extreme temperatures. Some seriously wounded men receive as many as five or eight injections in a few days. Nearly half the injured soldiers need plasma injections, and most of these require more than one dose. The total number of plasma injections about equals the total number of wounded men, say doctors at the fighting front. In the South Pacific transfusions from fit men on the spot are risky because of the prevalence of malaria.

American military surgeons have emphasized the low mortality rate among wounded men in this war. One of the most important factors responsible for this fact undoubtedly is human plasma which has been made available through the voluntary blood donations of millions of patriotic Americans under the direction of the American Red Cross. Truly, human blood has been "Life Saver 1 in World War II."

C. I. T. NEWS

ADMISSION OF VETERANS TO C.I.T.

THE policy of admission of veterans who wish to pursue courses of study at the California Institute of Technology has recently been established. This policy is directed principally to those who are seeking entrance for the degree of Bachelor of Science in Engineering or Science. Those who wish to continue their studies in pursuit of graduate degrees will be held to the usual requirements of the Graduate School. The details for the establishment of special refresher courses for men who have their B.S. degree have not been completed. Recently a questionnaire was sent to graduates of the Institute to determine how many were interested in such courses and the subject matter desired. The result of this questionnaire will assist in the formulation of a policy.

Two forms of leaves of absence have been granted to students. Those men whose education was interrupted because of induction into the armed services have been