Blood Transfusions in Peace and War:
The Cohn Process

Abstract:

The onset of World War II necessitated a widespread, effective and easy to use transfusion material to remedy shock or circulatory failure due to infection or blood loss as the leading killer of wounded troops. Albumin was determined to be the most effective product. Edwin Cohn devised a highly useful process to purify albumin from plasma, the non-cellular, liquid component of blood. The process revealed other plasma proteins that could be used against a variety of diseases. Variations on Cohn's process increased efficiency and safety of preparing blood for effective use in medicine.

Introduction:

Historical Background of Blood Plasma Transfusion:

During World War I, one of the greatest problems field doctors faced was the medical phenomenon "shock." Observers characterized the symptoms as a complete circulatory collapse and organ failure due to the copious loss of blood or wound infection. Medical experts who visited frontline casualty centers recommended immediate blood transfusion to prevent the onset of hemorrhaging and shock. (Surgenor 93) Otherwise, uncontrolled bleeding or hemorrhaging leads to tissue damage due to toxic buildup, resulting in arterial and capillary weakening. Organ failure will result once the local blood supply is cut off or heavily tainted, causing death. (Shock, Wikipedia)

American research efforts for determining the optimum transfusion material were accelerated by the start of World War II in Europe and the impending involvement of the United States. Initially, the project's goal was to supply Great Britain with transfusion-grade human plasma and kits as part of the "Blood for Britain" program. Plasma is the liquid, non-cellular component of blood that includes water, sugars, ions, dissolved oxygen and carbon dioxide and proteins. Plasma can be dried and preserved in vacuum sealed bottles. A blood transfusion is given following dissolution of the plasma in

distilled water. (Surgenor 98; Blood Plasma, Wikipedia) Dried plasma replaced whole blood transfusion due to simpler packaging and its much longer shelf life, lasting for several months. However, the scale of modern warfare in Europe demanded huge plasma supplies that the American donor pool could not satisfy. A better and more efficient transfusion alternative for human plasma was actively sought by the National Research Council, a committee that provided scientific and technical advice to the government and military. While the supply problem of plasma was remedied early in 1942 following Pearl Harbor due to the massive blood-donor support for the war effort, the research and development for a human plasma replacement continued. (Surgenor 100; The Plasma Program, Chapter XI)

Cohn's Identification of Albumin as a Transfusion Alternative for Plasma:

In May 1939, Edwin Cohn, a renowned expert in proteins and protein separation techniques, was enlisted to determine if bovine plasma could be safe for human use. Using his fractionation method, Cohn identified three major protein classes in Bovine plasma: fibrins, globulins and albumin. (Surgenor 110) Since fibrin was known as a clotting factor or coagulant, it would impede the already weak circulation of wounded soldiers suffering from blood loss if applied directly into the circulatory system. Cohn knew that both globulin and albumin had the effect known as oncotic pressure of blood. It is the hydrostatic pressure of large plasma proteins, such as albumin and globulin, on blood vessel walls. This is a natural pressure that balances the diffusion of smaller ions and water and is vital in keeping blood vessels turgid to allow adequate blood circulation. However, globulin or albumin should not be applied to wounded and dehydrated soldiers because the proteins would then block the blood vessels if there is insufficient flowing fluid. Thus, plasma would be necessary in place of globulin and albumin because of the fluid it provides from its dissolution in distilled water. For soldiers suffering from blood loss without significant dehydration, these proteins would be critical for recovery by supporting the circulatory system. (Serum Proteins, Oncotic Pressure, Wikipedia)

From tests on volunteers with an amount of blood taken, albumin was more successful than globulin due to faster recovery rate and fewer allergenic reactions. In September 1941, using larger amounts of bovine albumin, Cohn conducted trials to test if albumin was more effective than plasma in treating blood loss. In this experiment, he injected albumin in a 20% salt solution directly to the volunteers, without dilution in water. Cohn discovered that dried bovine albumin was immediately effective without added distilled water; patients generally recovered in the same time or faster than with plasma. However, one patient died from severe allergenic reactions. This put the bovine albumin program on hold, but Cohn's simultaneous projects on the separation of human albumin continued. His tests also revealed that human albumin was stable between -45 to 45 degrees Celsius. Furthermore, albumin's smaller packaging required less space than the plasma bottles and did not require extra water for dilution prior to application. In 1942, human albumin was officially sanctioned by the NRC as a suitable alternative for plasma for wounded soldiers not suffering from serious dehydration. (Surgenor 130-160)

Cohn's Method to Separate Albumin from Plasma:

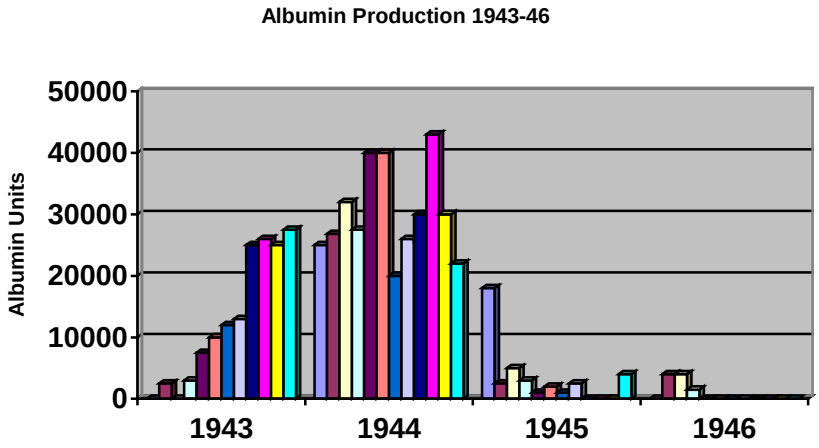
Before the human/bovine albumin project, Cohn had devised an effective fractionation procedure to separate proteins. This process was successful for separating albumin from blood plasma. It involved chilled ethanol that initially recovered ~85% of the albumin in whole blood, much larger than that achieved in previous fractionation processes for albumin. Once Cohn received official approval from the NRC for the use of human albumin as an alternative to plasma, his separation process was licensed to medical and biologic firms that provided medical supplies to the Allies in World War II. With the Cohn Process, medical albumin was manufactured in significant amounts from 1943 onwards to augment supplies of plasma. Albumin was extensively used in the Pacific Theater of World War II and the Korean War; it saved many lives under conditions where the use of plasma would have been time-consuming and dangerous (application under enemy fire). Albumin packages could be used by wounded soldiers without the need for a medic. (Surgenor 203, 215-235; The Cohn Process, Wikipedia)

Other Plasma Proteins and Hepatitis:

In the latter years of World War II, to treat wounded soldiers, Cohn advocated using thrombin and fibrin foam, byproducts of the Cohn Process in retrieving albumin. Fibrin foam and thrombin are clotting agents that slow and stop bleeding by forming a protein mesh over wounds. These plasma proteins increase aid options for troops and medics. Both thrombin and fibrin foam were accepted by the NRC in Spring 1944. The supplies of albumin, thrombin and fibrin foam helped Allied troops at a pivotal time during the vicious battles in the Pacific and also the Russian troops, who were incurring heavy casualties while pushing into Poland and Germany.

Cohn's tests with globulin, another plasma protein separated in the Cohn Process, was effective in treating measles in 77% of infected patients, while 22% showed only mild symptoms. This result was especially useful for treating children, who are most susceptible to measles.

Many troops who received albumin or other blood transfusions developed jaundice or yellowing of the skin and eyes. It was attributed to Hepatitis A, which contaminated certain transfusion supplies. Cohn's associates Neefe and Stokes found that globulin was able to modify the outer structure of the Hepatitis A virus; globulin either inactivated the virus or decreased its virulence. Cohn also modified his process to deactivate the hepatitis virus by preheating the plasma to 68 degrees Celsius before separating the plasma proteins from plasma. Globulin and Cohn's heated fractionation saved the lives of many soldiers and patients suffering from Hepatitis A and measles. (Surgenor, 220-235)

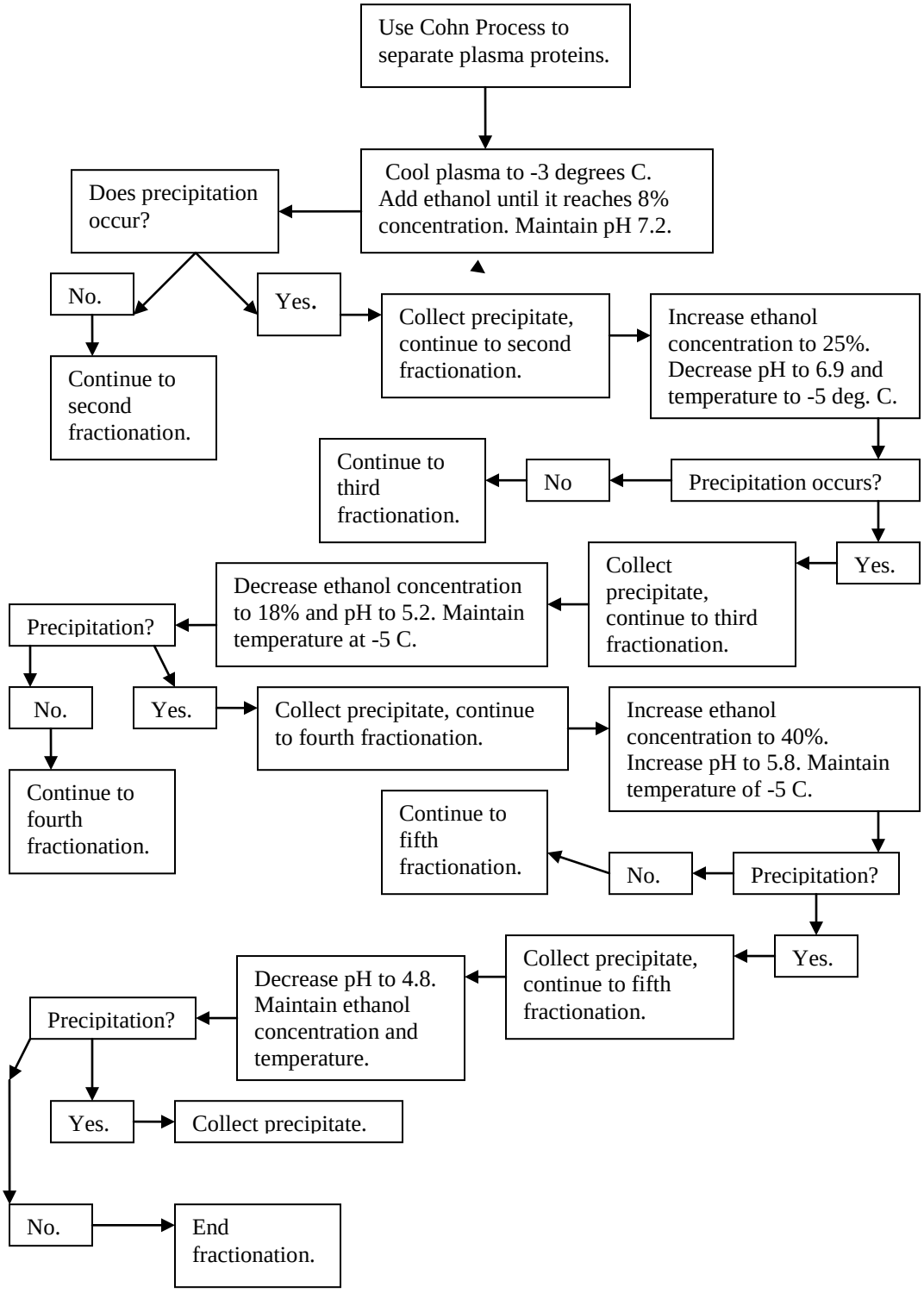


The Process:

The Cohn Process separates proteins from plasma in five fractions. The separation depends upon the different solubilities of the various proteins under varying conditions of pH, temperature and ethanol concentration. When conditions are suitable, specific protein(s) will precipitate and can be collected as a fraction of the total plasma. During the process, the concentration of ethanol increases from 8 to 40% in the plasma solution, the pH decreases from neutral to 4.8 and the temperature falls from 72 to -5 degrees Celsius.

The five fractions are numbered in the order of collection due to protein precipitation. Fraction I collects the precipitation with most of the fibrinogen (precursor to fibrin foam) proteins in plasma. Fractions II and III includes most of the thrombin and some of the globulin proteins. Fraction IV includes the remnant of globulins. Fraction V contains most of the albumin component of plasma. Roughly 15-20% of the total albumin concentration in plasma is found in the fractions before the fifth fraction. (Surgenor 120-135; Cohn Process, Wikipedia)

Flow Chart


Use Cohn Process to separate plasma proteins.
Cool plasma to -3 degrees C. Add ethanol until it reaches 8% concentration. Maintain pH 7.2.
Does precipitation occur?
No.
Yes.
Continue to second fractionation.
Collect precipitate, continue to second fractionation.
Increase ethanol concentration to 25%. Decrease pH to 6.9 and temperature to -5 deg. C.
Precipitation occurs?
No
Continue to third fractionation.
Yes.
Collect precipitate, continue to third fractionation.
Decrease ethanol concentration to 18% and pH to 5.2. Maintain temperature at -5 C.
Precipitation?
No.
Yes.
Collect precipitate, continue to fourth fractionation.
Increase ethanol concentration to 40%. Increase pH to 5.8. Maintain temperature of -5 C.
Continue to fourth fractionation.
Precipitation?
No.
Continue to fifth fractionation.
Yes.
Collect precipitate, continue to fifth fractionation.
Decrease pH to 4.8. Maintain ethanol concentration and temperature.
Precipitation?
Yes.
Collect precipitate.
No.
End fractionation.

Conditions for Fractionation

Fraction:	1	2	3	4	5
% Ethanol Concentration in Plasma:	8	25	18	40	40
pH:	7.2	6.9	5.2	5.8	4.8
Temp. (C):	-3	-5	-5	-5	-5
Fraction of total Plasma Volume collected as Precipitation (%):	5.1	3	3	3	1

Consequences:

Due to Cohn's Process, dried albumin was mass produced and shipped with medical kits alongside dried plasma. The albumin supplies were produced at a time when the Allies were sustaining heavy casualties between 1943 and 1945. Dried albumin was relied upon in World War II during D-Day, the Pacific Campaign and later, the Korean War. Albumin saved many lives from hemorrhaging and shock due to its quick application.

Initially, Cohn only intended to use his process for albumin separation to produce a plasma alternative. However, he discovered in the fractions other plasma proteins that had a variety benefits, especially as disease and infection treatments. First, globulin could treat both measles and Hepatitis A by boosting the immune system. Fibrinogen and thrombin could be converted to fibrin foam, which was an effective clotting agent. Globulin became essential in treating children with measles or Hepatitis A, while soldiers

relied on fibrin foam as a quick-acting clotting agent to stop heavy bleeding. Modern fibrin sealant is a modification of fibrin foam and is used in most surgical operations as an adhesive glue to stop bleeding. A subclass of globulins, the gamma globulin, was later recognized to suppress the autoimmune response against foreign blood cells, which occurs during blood transfusion. This is still given to patients suffering from an immune reaction to donor blood. (Fibrinogen, Globulins, Gamma Globulins, Wikipedia)

Modifications to the Cohn Process reduced ethanol usage, sterilized plasma and plasma protein supplies through heating, and offered purification techniques. Presently, the Cohn Process remains the basic process in plasma purification and plasma isolation for most blood processing companies. Its use has spread to developing countries, where the need to prevent blood-borne infections is vital for survival under unsanitary conditions and malnourishment. Hepatitis A is especially rampant among societies where communal interactions in an unsafe and unsanitary manner are common. Cohn's Process would at least remove some of the danger through plasma or blood protein exchanges by inactivating Hepatitis A through heating or using separated globulin proteins. (Cohn Process, Wikipedia)

Conclusion:

The Cohn Process saved lives in World War II by facilitating the rapid production of albumin to complement plasma supplies. Simultaneously, the process also revolutionized the blood processing industry, offering a wealth of tools and knowledge into the use and composition of various blood constituents. Discoveries made through the process included a better understanding of transfusion contamination and methods to prevent them. It provided new insights into different uses of blood proteins such as the clotting aid, fibrinogen. These discoveries have extended the use of the Cohn Process beyond military medical supplies and into the civilian health services around the world. Modern blood processing firms still use variations of the Cohn Process that serve as the foundation for transfusion product processing and purification. Applications derived from the Cohn Process have had an immeasurable effect on society by expanding the medical

use of blood, safeguarding against transfusion infections and increasing the accessibility of purified medical blood products.

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