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# 3,041,242
# GAS AND HEAT STERILIZATION OF DRIED HUMAN PLASMA

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Human blood plasma found extensive use during World War II as a natural blood expander. Its use was discontinued during the Korean war due to the fear of the presence therein of the virus of homologous serum hepatitus. Since that time many efforts have been made to provide a process whereby this virus in normal human blood plasma could be inactivated to render the plasma positively safe for both military and civilian use. Heretofore, these efforts have not been successful. An important object of the present invention is, therefore, to provide a process for the complete destruction of any virus of homologous serum hepatitus which might be present in normal human blood plasma.

The fibrinogen fraction of whole plasma is separated from the plasma and made up into pads for checking hemorrhages, particularly after surgery. However, this fibrinogen is subject to the same disadvantage as the whole plasma in that it may contain the same virus. Accordingly it is another object of this invention to provide a process for the treatment of fibrinogen to inactivate the virus of homologous serum hepatitus contained therein.

A broader object of the present invention is to provide a process for the inactivation of bacteria and viruses in proteins and other materials intended for administration to humans.

Other objects and advantages of this invention it is believed will be readily apparent from the following detailed description of preferred embodiments thereof.

Briefly, this invention comprehends within its scope the discovery that the virus of homologous serum hepatitus in normal human blood plasma and/or the fibrinogen thereof can be fully inactivated by first heating the dried plasma or fibrinogen under conditions such as to attenuate or weaken the contaminants including the above-mentioned virus, followed by subjecting the material to the action of a lethal gas to kill the contaminants. The plasma and fibrinogen so treated is unchanged in any way, except for the destruction of the contaminants. It must be emphasized that the process of this invention requires that the material being treated be initially in the dried condition. The heat treatment of liquid plasma (at 60° C. for 10 hours) has heretofore been found to result in inactivation of the virus, but it is not practicable for use since the heating of the plasma in the liquid state causes denaturization of certain of the plasma proteins, rendering it unfit for use.

In carrying out the process of this invention, the dried proteinaceous material is first heated at a temperature and for a length of time sufficient to result in attenuation or weakening of the contaminants, which may include the virus of homologous serum hepatitus. It has been found that a definite temperature-time relationship exists in that the temperature required varies inversely with the time period of treatment. It has been further found that a temperature of at least about 37.5° C. is required for attenuation, and that the temperature should be kept below about 70° C. to prevent denaturization. Several days exposure are required for the lower temperatures, whereas only several hours are required for the higher temperatures. From the standpoint of commercial practicality and complete safety the preferred attenuation



conditions are a temperature range of 50°–70° C. and a minimum time of exposure of about 10 hours.

Following the attenuation step, the material is subjected to the action of a lethal gas. The preferred gas is ethylene oxide, which should be applied under high vacuum conditions to eliminate any possibility of contact of the gas with static electricity. Moreover, the high vacuum permits maximum utilization of the lethal gas, both from the standpoint of speed and killing effectiveness inasmuch as the absence of air and other oxidizing media permits quick and ready penetration of the gas into the plasma. During the gassing operation under high vacuum conditions it is necessary to heat the vacuum chamber to prevent freezing of the plasma. Preferably the temperature at this stage is maintained above about 100° F. Any gas which is lethal to the virus of homologous serum hepatitus, or to the particular bacteria or virus to be destroyed, may be used. By way of example, such other gases may include ethylene oxide and carbon dioxide mixtures, sulfur dioxide, formaldehyde and the like.

The following specific examples are illustrative of the process of the present invention but it is to be understood that the invention is not to be limited to the details thereof:

*Example 1*

A number of fresh human bloods were centrifuged, the plasmas drawn off and pooled. The pool was clarified through a De Laval and the clarified pool divided into two lots, one of 25 containers of 300 cc. each for processing in accordance with this Example 1, and another of 13 containers of 300 cc. each for processing in accordance with Example 2 below. Both lots were immediately shell frozen and three frozen containers of the first lot were held in —20° C. storage without further processing for later comparative testing.

The first lot was dried in the conventional manner for preparing dried human blood plasma. Two containers of the dried material were stoppered under vacuum, sealed and set aside for later test. The remainder of the lot were stoppered under atmospheric pressure and sealed. They were then heated in a water bath at 60° C. for ten hours.

The seals and stoppers were then removed from the containers and replaced with caps which permitted the passage of gases. The containers were placed in a steam-jacketed vacuum chamber under 28 inches of vacuum. Ethylene oxide was admitted into the chamber until the vacuum was reduced to 25 inches. The vacuum chamber was then closed off and steam was admitted to the jacket. The temperature in the vacuum chamber was thus brought up to 110° F. and maintained at that temperature for 15 hours. At the end of this time, air was admitted into the vacuum chamber through filters until the pressure was equalized with atmospheric pressure.

The containers and their contents were removed from the vacuum chamber and placed in a vacuum drying chamber under a vacuum of 400 microns. Dry nitrogen was then admitted and allowed to equalize the vacuum to normal atmospheric pressure. The evacuation and nitrogen washing operation was repeated three times whereupon the lot was removed from the drying or washing chamber and stoppered under vacuum.

Electrophoretic pattern, hemoglobin, pyrogen, sterility and other tests of the product showed it to be unchanged from the untreated material, yet sterile and safe for use. The dried plasma product is readily reconstitutable for use in the same manner as conventional dried plasma.

*Example 2*

The process of this example was identical to that of

Example 1, except that here the plasma, comprising the second lot referred to in Example 1, was irradiated under ultra violet light in accordance with conventional practice prior to filling into the containers and freezing. The product exhibited no differences over that of Example 1.

In addition to its use in the treatment of human blood plasma, the process of this invention is applicable to the treatment of plasma fibrinogen, other proteins such as, for example, bovine albumin, gamma globulin, and the like. Moreover, the process can be used in the treatment of vaccines to destroy viruses and other microorganisms therein. The process has been experimentally applied to plasma-microorganism mixtures comprising mixtures of plasma and poliomyelitus (Lansing strain), plasma and *A. acrogenes,* and plasma and *B. subtilis* var. *globigii* spores, with complete destruction of the microorganisms. The process conditions in the treatment of these three experimental mixtures were the same as in Example 1, except that the ethylene oxide gas sterilization step was carried out overnight at 43° C.

Having fully described our invention, it is to be understood that we do not wish to be limited to the details set forth, but our invention is of the full scope of the appended claims.

We claim:

1. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature and for a length of time sufficient to attenuate the virus, and then applying a lethal gas under high vacuum conditions to the dried plasma to kill the virus.

2. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature between about 37.5 and 70° C. for a length of time sufficient to attenuate the virus, and then applying a lethal gas under high vacuum conditions to the dried plasma to kill the virus.

3. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature of 50–70° C. for at least about 10 hours to attenuate the virus, and then applying a lethal gas under high vacuum conditions to the dried plasma to kill the virus.

4. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature of 60° C. for about 10 hours to attenuate the virus, and then applying a lethal gas under high vacuum conditions to the dried plasma to kill the virus.



5. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature and for a length of time sufficient to attenuate the virus, and then applying ethylene oxide under high vacuum conditions to the dried plasma to kill the virus.

6. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature of 50–70° C. for at least about 10 hours to attenuate the virus, and then applying ethylene oxide under high vacuum conditions to the dried plasma to kill the virus.

7. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum hepatitus contained therein, comprising the steps of heating the dried plasma at a temperature of 60° C. for about 10 hours to attenuate the virus, and then applying ethylene oxide under high vacuum conditions of the dried plasma to kill the virus.

8. A process for the treatment of dried normal human blood plasma to inactivate any virus of homologous serum heptatitus contained therein, comprising the steps of heating the dried plasma at a temperature and for a length of time sufficient to attenuate the virus, then applying a lethal gas under high vacuum conditions to the dried plasma to kill the virus, and washing the material free of said lethal gas by the application of an inert gas.

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