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PROTEIN FRACTIONATION CENTRE
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Summary of Meeting

Date and Place: September 9, 1982
Office of Biologics

Subject: Heat Treatment of Plasma Products

Purpose: This meeting was held to discuss certain technical problems with regard to heat inactivation of viruses, particularly non-A, non-B hepatitis virus in plasma derivatives.

The principles of heat inactivation of microorganisms were reviewed by Dr. David Kosow (American Red Cross). From currently available information on the inactivation of other microorganisms (in most cases bacteria), it can be concluded that viral inactivation by heat is the result of the single denaturation of a single protein of the organism. There is invariably a very strong temperature dependence with inactivation rate increasing by 10 fold with less than a 10 °C temperature rise.
Dr. Kosow pointed out the logarithmic nature of the inactivation process. It follows from this that the residual infectiousness of a material is directly related to the infectious level of the treated material.
Dr. Purcell pointed out that viruses, even those within the same virus class can differ by several orders of magnitude in their susceptibility to inactivation.

Dr. Ken Ingham reviewed the physical biochemistry of protein stabilization. Four classes of compounds have been used to stabilize proteins; specific ligands, neutral salts, neutral sugars and acid sugars. While heat inactivation of some small model proteins is reversible the plasma proteins seem to be irreversibly denatured and aggregated. Studies of the concentration dependence of plasma protein denaturation indicate that this is due to monomer formation, the denatured monomer then rapidly and irreversibly aggregating, i.e., aggregation is the result of denaturation and is not the cause of the denaturation.

The contamination of blood products with the transmissible agent(s) of non-A, non-B hepatitis can lead to chronic liver disease. Unfortunately, the characterization of this/these agents is minimal and chimpanzee experiments are slow and fraught with inherent problems. Dr. Edward Tabor described studies of the inactivation of non-A, non-B hepatitis which have been conducted so far. One hundred chimpanzee infectious doses per ml of the agent contained in Inoculum I have been shown at NCDB to be susceptible to inactivation by heating at 60 °C for 10 hours in the presence of 10% serum. This agent has also been shown to be inactivated by formalin even in the presence of substantial amounts of protein in studies conducted in the United States (NCDB) and in Japan. In order to perform an adequate inactivation experiment, Dr. Tabor felt that it was mandatory to have a titered inoculum. Any product to which this inoculum was added would have to be free of immunoglobulins in order to prevent possible immune inactivation. Any animal model used would have to be reproducible. While sporadic non-A, non-B infection has been achieved in a few marmosets, only the chimpanzee fulfills the criteria for a reproducible animal model.

Any chimpanzee used should be colony born. With such chimpanzees non-A, non-B disease can be achieved in the majority of instances, whereas with wild-caught animals the apparent success rate is much lower. Each animal infused with inactivated virus who does not develop evidence of disease should be inoculated with control material to establish the susceptibility of the given animal.

Available characterized inocula for non-A, non-B hepatitis are in limited supply and, with a single exception, of fairly low titer. An inoculum with an infectivity titer lower than 10^2 CID_{50} would not be useful for any inactivation studies.

The identity of the agents in the different experimental non-A, non-B inocula used in these experiments is not established. Most chimp studies of non-A, non-B have been done with samples from patients treated with blood rather than fractionated products and clinical evidence would suggest that these may be two different agents. Cross challenge experiments are fraught with problems of interpretation even if the second challenge is positive. The overall data indicate several forms of non-A, non-B hepatitis exist.

Discussion of the inocula then revolved around whether to use a common inoculum on the assumption that the available inocula contain an agent of significance, or one to use multiple inocula hoping to gain more information about the variety of inocula available. The ultimate, if not enthusiastic, consensus was that it was probably better to have a single inoculum for any non-A, non-B inactivation studies.

A critical point discussed was whether heat inactivation studies of non-A, non-B hepatitis should be done at this time. A critical part of this decision is whether or not one would change an inactivation process known to inactivate hepatitis B because of apparent failure to inactivate non-A, non-B. Several producers of AHF felt that they would, in fact, make adjustments to a procedure known to produce products free of hepatitis B if chimpanzee experiments indicated that non-A, non-B hepatitis was still surviving.

In light of the above discussion of the difficulties of studying the inactivation of non-A, non-B inactivation in plasma products, it was suggested that representatives of the Office of Biologics and the NHLBI try to coordinate such studies, e.g., establish a common inoculum, identify colony born chimps, etc. Dr. Barbosa pointed out that this type of study was a lower priority for NHLBI than studies to establish diagnostic tests.

Several participants suggested that the use of marker viruses, i.e., viruses more easily measured than the hepatitis viruses, might be useful. One could possibly use a panel of such viruses in parallel with some chimp studies with hepatitis to more rapidly and easily evaluate various procedures to produce safer products. Three viruses related to hepatitis B have been studied--ground squirrel, woodchuck and duck. All of these have their own problems. No consensus could be reached on the usefulness of this approach.

Hepatitis safety of such treated products might also be established by clinical trials in certain cases. One such approach involves clinical trials of acquired deficiencies (this is not an approved use but estimates would indicate a significant amount of use). Following chimpanzee studies, it would still be desirable to have surveillance of high risk plasma products particularly hemophiliacs.

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