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IMMUNOLOGIC EVALUATION OF ITALIAN HAEMOPHILIACS TREATED WITH COMMERCIAL CONCENTRATES. L.Rossi (1), A.M.Giangregorio (2), S.Bartolai (1), L.Lecchini (3), M.Bendinelli (2), F.Panicucci (1). Haemophilia Centre (1), Institute of Virology (2), Institute of Statistical Sciences (3), University of Pisa, Pisa.

The presence of the antibody to human immunodeficiency virus (HIV) was investigated in 487 serum samples withdrawn from 216 haemophiliacs (186 haemophiliacs A and 30 B) who came to the Pisa Centre between 1977 and 1986. Results show that a considerable proportion of haemophiliacs (15%) were already positive in 1982. In haemophiliacs A, who were treated up to 1983 with concentrates made from US plasma, but from 1984 on began to use concentrates made from Italian plasma, this proportion in 1986 was 16%. In haemophiliacs B who have continued using concentrates from US plasma, the prevalcence rate of Anti-HIV was found to be much higher (63%). As regards the contact with the virus of hepatitis B (HBV), antibodies were found in 64% of haemophiliacs and the viral antigens in 6%. 137 of these haemophiliacs (112 A and 25 B) were examined between 1985 and 1986 for index of hepatic cytolisis, immunological and clinical status. In 65% of haemophiliacs the level of serum glutamic pyruvate transaminase (SGPT) was mildly increased, perhaps for the presence of chronic hepatitis. In Anti-HIV negative subjects we found a decrease of T helper/T suppressor (Th/Ts) ratio, with a mean of 1.3 (in controls, 1.7), due especially to an increase of Ts (mean 0.8 X 10^3/cu.mm.; in controls, mean 0.6). Also in Anti-HIV positive haemophiliacs there was a decrease of Th/Ts ratio (mean 1.1), but this was mainly due to a decrease of Th (mean 0.7 X 10^3/cu.mm.;in controls, mean 1.1). Clinical evaluation of the Anti-HIV positive subjects showed 2 patients with AIDS related illness with opportunistic infections and 13 haemophiliacs (33% of seropositive subjects) with one or more of these abnormalities: thrombocytopenia, lymphoadenopathy, slight persistent fever, diarrhea.

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INACTIVATION OF HEPATITIS VIRUSES AND HIV IN PLASMA AND PLASMA DERIVATIVES BY COMBINED TREATMENT WITH ß-PROPIOLACTONE/UV-IRRADIATION. W. Stephan (1), H. Dichtelmüller (1), A. M. Prince (2), L. Gürtler (3), F. Deinhardt (3). Biotest Pharma, Frankfurt/Main, FRG (1), New York Blood Center, N. Y., U.S.A. (2) and Max von Pettenkofer Institut, München, FRG (3).

A combined treatment of plasma and plasma derivatives by ß-Propiolactone (ß-PL)/UV-irradiation is in use at Biotest for the preparation of the virus safe serum preserve Biseko® and coagulation factor concentrates.

The efficacy of this sterilization procedure has been demonstrated for HAV (> 8.2 $\log_{10}$), HBV (7.0 $\log_{10}$) and HNANB (> 4.5 $\log_{10}$). As HIV has become a major problem the inactivation of HIV by ß-PL/UV in human plasma was tested. Pooled human plasma was spiked with $10^{4.2}$ infectious units per ml of the Gallo strain of HIV/HTLV-III and sterilized with 0.25 % ß-PL, 60 min at pH 7.2 and subsequently UV-irradiated (4 x 20 W). After treatment with ß-PL alone or ß-PL/UV no infectious HIV was detectable by reverse transcriptase assay in inoculated H-9 cultures after 14 days of cultivation (> 4.2 $\log_{10}$ inactivation). When the virucidal efficacy of ß-PL and UV was tested separately, ß-PL inactivated > 3.5 $\log_{10}$, UV-irradiation another 2.5 $\log_{10}$ of HIV, as demonstrated by immunofluorescence tests in H-9 cultures 27 days after inoculation.

When cryoprecipitate/F VIII-concentrate was sterilized by ß-PL and UV, > 4.5 $\log_{10}$ of HIV were inactivated by UV and > 3.5 $\log_{10}$ by ß-PL. The results indicate, that the combined treatment by ß-PL/UV inactivates all potential titers of HIV, which can be expected in screened and pooled human plasma or cryoprecipitate, used for the preparation of virus safe plasma derivatives.

Table Inactivation of HIV in plasma and cryoprecipitate/F VIII by ß-PL and UV

Preparation/treatment	inactivation ($\log_{10}$)
HIV + Plasma + ß-PL/UV	> 4.2
HIV + Plasma + ß-PL	> 3.5
HIV + Plasma + UV	2.5
HIV + Cryo + ß-PL	> 3.5
HIV + Cryo + UV	> 4.5

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VIRUS SAFETY OF ANTITHROMBIN III CONCENTRATE KYBERNIN P - A PROSPECTIVE CLINICAL TRIAL. Clemens R, Wirl G, Velten C, Röthig HJ. Clinical Research Department, Behringwerke AG Marburg, West Germany.

In 13 healthy male volunteers a prospective clinical trial was performed to evaluate virus safety of the antithrombin III concentrate Kybernin P in regard to hepatitis B, non-A/non-B and HIV transmission. As the volunteers were participants of a pharmacokinetic study they received either a fixed dosage of 1000 units Kybernin P as bolus injection or a dosage of 50 units per kg body weight as short-term infusion. Two different batches of Kybernin P were used.

Whereas in all 13 volunteers virus safety in hepatitis non-A/non-B and HAV transmission could be monitored, only those volunteers who were not vaccinated (n=3) against hepatitis B or who had no protecting antibodies of anti-HB_stype despite vaccination (n=3) were to be included in the hepatitis B monitoring.

All 13 volunteers were followed-up for 1 year according to the standards of the International Committee on Thrombosis and Hemostasis (ICTH). For detection of a potential hepatitis non-A/non-B transmission transaminases (AST, ALT) were determined in biweekly intervals during the first 6 months of the observation period and thereafter in monthly intervals. Hepatitis B seromarkers as well as anti-HIV were assessed bimonthly. Furthermore, all volunteers were clinically examined at every follow-up.

None of the 13 volunteers revealed an increase of transaminases to the 2.5 fold of the upper normal level which is considered to be the borderline level for hepatitis non-A/non-B diagnosis. Furthermore, in none of the volunteers a seroconversion for hepatitis B or HIV could be detected. Thus, Kybernin P is to be considered as hepatitis B, hepatitis non-A/non-B and HIV safe.

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INACTIVATION OF VIRUSES IN PLASMA ON TREATMENT WITH TRI(N-BUTYL) PHOSPHATE (TNBP) DETERGENT MIXTURES. **M.P.J. Piët, S. Chin, A.M. Prince, B. Horowitz. The New York Blood Center, New York, New York, U.S.A.**

Treatment of plasma with organic solvent/detergent mixtures at the time of plasma collection or plasma pooling could reduce the exposure of technical staff to infectious virus and enhance the viral safety of final product. Treatment of plasma for 4 hours with 2% TNBP at 37°C or with 1% TNBP and 1% Tween 80 or Triton X-45 at 30°C resulted in the rapid and complete inactivation of $\geq 10^4$ tissue culture infectious doses (TCID-50) of vesicular stomatitis and Sindbis viruses, used as surrogates. TNBP and TNBP/Tween treatment of plasma was shown to inactivate $\geq 10^4$ TCID-50 of human immunodeficiency virus. TNBP treatment of plasma contaminated with 10^6 chimpanzee infectious doses (CID-50) of HBV and 10^5 CID-50 of NANBHV prevented the transmission of hepatitis to chimpanzees through 6 months follow-up.

Immediately following treatment with 2% TNBP, the recovery of AHF, factor IX, factor V, and antithrombin III was 75%, 90%, 65% and 100%, respectively. A ≥90% recovery of AHF was observed with TNBP/detergent mixtures. Treated plasma was fractionated into AHF and prothrombin complex concentrates, immune globulin, and albumin by published techniques. Prior treatment with TNBP or TNBP and detergent did not affect the separations of desired proteins. Therefore, it appears possible to inactivate viruses in plasma prior to execution of standard fractionation procedures. If desirable, products prepared from TNBP-treated plasma can be subjected to additional procedures to further insure virus safety.