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A LOW pH PREVENTS DENATURATION OF HEAT TREATED ANTI-HAEMOPHILIC CRYOPRECIPITATE. O.H. Skjønsberg (1), K. Gravem (1), P. Kierulf (2), H.C. Godal (1). Haematological Research Laboratory (1) and Central Laboratory (2), Ullevål Hospital, University Clinic, Oslo, Norway.

The AIDS epidemic has necessitated heat treatment of FVIII:C products in order to inactivate HIV-virus. When antihaemophilic cryoprecipitate (CP) is heated (68°C, 24 hours), the solubility and the concentration of FVIII:C, von Willebrand factor and fibrinogen are substantially reduced. Such heat denaturation is prevented by addition of synthetic amino acids (Syntamin 17) prior to lyophilization and heat treatment (Ref.:Margolis and Eisen, Skjønsberg et al.). The exact mechanism of this protective effect is not known, but in our opinion, the large buffering capacity of Syntamin is of major importance. Thus, in the presence of Syntamin (4 mg/unit FVIII:C), a pH of 7.4 was maintained during lyophilization and heat treatment, while in ordinary CP, pH rose from 7.8 to 8.6 during the same procedure. Moreover, heat denaturation of CP was prevented by replacing Syntamin with a phosphate buffer, keeping a stable pH below 7.4. It is of interest to notice that apart from the effect on pH, we were unable to observe any influence of Syntamin on heat denaturation of plasma proteins in solution, on degradation of FVIII:C and fibrinogen by thrombin or plasmin or on the solubility of fibrin in plasma and CP.

Heat treatment of CP at various pHs indicated that the best product was obtained when pH was kept between 6.5 and 6.8. By using an acidic buffer instead of Syntamin, the disadvantages of the latter, such as increased residual moisture leading to discolouration, a probable stabilizing effect on virus and increased costs, may be avoided.

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IMMUNOCHEMICAL ANALYSIS OF FACTOR VIII (FVIII) IN PLASMA AND HEAT-TREATED CONCENTRATES. S.J. Edwards, G. Kemball-Cook and T.W. Barrowcliffe. National Institute for Biological Standards and Control, London, U.K.

Using the method of Weinstein et al. (Proc.Natl.Acad.Sci. USA, 78, 5137-41, 1981) the FVIII polypeptide distribution in wet and dry heated concentrates, a monoclonal-purified concentrate and fresh plasma was examined. Samples were incubated for 2 hours at 37°C (in the presence of polyethyleneglycol 4000 to aid complex formation) with ^{125}I-Fab' fragments prepared from a polyclonal human anti-FVIII:C antibody. The complexes were electrophoresed in a 3-9% polyacrylamide gradient gel, in the presence of SDS, under non-reducing conditions and visualised by autoradiography.

Fresh plasma showed a range of peptide bands of apparent M.Wt. 80-280 kD, with a major band at 280 kD. FVIII concentrates showed a similar range of bands and, for one manufacturer's product (product E), an additional strong band of 40-50 kD. The proportion of total FVIII antigen in the 280 kD band was estimated by densitometry to be 20-40% in concentrates, compared with 65% in fresh plasma. Severe haemophilic plasma had no bands, confirming the specificity of the technique. FVIII antigen in 'wet' heated concentrates was shown to be more degraded (increase in low molecular weight forms) than in dry heated concentrates.

Fresh plasma incubated at 37°C for 24 hours showed increased amounts of FVIII antigen in a low molecular weight form (90 kD).

Treatment of concentrates and plasma with thrombin resulted in a change of the peptide band pattern, which was dependent upon thrombin concentration and incubation time. Loss of the 280 kD band and intensification of a 90 kD band was observed, which correlated with an increase in FVIII:C by one-stage assay. Further proteolysis resulted in a band of inactive material of 40-50 kD, with identical mobility to the band seen in product E. FVIII:C activity in product E was higher by one-stage than by two-stage assay, and these results suggest more extensive thrombin degradation in this product.

The results show that the molecular form of FVIII in concentrates is dependent upon storage of plasma, the method of concentrate preparation and the type of heat treatment.

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BLOOD BANK PRODUCTION OF HIGH YIELD, HIGH PURITY, HEAT TREATED F VIII CONCENTRATE FROM HEPARINIZED BLOOD. K. Wallevik, J. Ingerslev and S. Stenbjerg Bernvil. Department of Clinical Immunology, Aarhus Kommunehospital, DK-8000 Aarhus C, Denmark.

Applying trivial blood bank equipment we have developed a method to produce a heat-treated factor VIII concentrate from heparinized blood with an over-all yield between 40 and 50%, a specific activity around 2 i.u./mg and a low fibrinogen content (3-4 g/l). Electrophoretic multimer analysis of the von Willebrand factor reveals a band pattern close to that of native plasma. The in vivo recovery is 99±25% and t/2 11.4±2.4 h (n=5). The method includes 3 precipitations in commercial "blood-bag" systems: (1) The classical cryoprecipitation, (2) Precipitation at 0°C of "cold insoluble globulins" including F VIII/vWf, (3) Precipitation of non F VIII/vWf related proteins at 10°C, pH 7.0 and specified ionic conditions.

The F VIII rich supernatant is stabilized by amino acids and citrate, freeze dried, and heated to 68°C for 24 h.

In our blood bank with ~40,000 donations per year we have in a year produced ~1,4 mill i.u. of the High Purity F VIII concentrate. This means that around 16,000 donations per year are collected in heparin, and that F VIII depleted plasma which we deliver for clinical use, either as liquid plasma or as a freeze-dried product, contains solely heparin (~4 i.u./ml) as anticoagulant.

The method is technically simple which together with the high yield and high purity of the F VIII concentrate makes the production economically profitable. The method is applicable in areas with limited technical resources.

We find it also important that F VIII concentrate, manufactured locally from healthy voluntary donors with personal attachment to their blood bank reveals the lowest risk for transmission of diseases to the patients.

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ANTI-HIV AFTER HEATED CLOTTING FACTOR CONCENTRATES IN HEMOPHILIACS. G. Mariani*, A. Ghirardini*, P. Verani**, F. Mandelli*, G.B. Rossi**, P.M. Mannucci*** and S. Buttò**. *Dept. Hematology, Univ. of Roma "La Sapienza"; **Dept. Virology, Istituto Superiore Sanità, Roma;***A. Bianchi Bonomi Hemophilia & Thrombosis Center, Univ. of Milano, Italy.

In Italy, heated concentrates became the only source of hemophilia therapy since July 1985, when a government act enforced their use instead of nonheated concentrates. Since then 63 anti-HIV seronegative hemophiliacs treated with heated concentrates were followed-up prospectively, focusing on the development of anti-HIV. Anti-HIV (documented by persistent positivity for ELISA and WB) occurred in 6 patients who had no other risk factor for HIV infection. For 3, anti-HIV was first found in Sept., Oct. or Nov. 1985 i.e. within 4 months of the last infusion of unheated concentrates (July 1985). For another patient, anti-HIV was found in Sept. 1986, but no other sample was available after the last negative test (Nov. 1985). For these 4 cases, therefore, we cannot exclude that seroconversions are due to nonheated concentrates used until July 1985. For 2 patients, however, anti-HIV occurred in July 1986, i.e. 11 months after change to heated concentrates. For both a hemophilia A patient (treated exclusively with a concentrate dry-heated for 72 hr at 68°C) and a hemophilia B patient (treated with both a steam-heated concentrate and a concentrate dry-heated for 72 hr at 68°C) the last seronegativities were found in March 1986, i.e. 7.0 and 7.5 months after commencing the use of heated concentrates or 3.5 and 4.0 months before the first seropositivity. The overwhelming majority of heated concentrates were prepared from non-donor-screened plasma. In conclusion, two anti-HIV occurred in previously seronegative patients treated exclusively with heated concentrates. Intensity and duration of concentrate exposure to heating were greater than those for the commercial dry-heated concentrate (60°C for 30 hours) that caused two reported seroconversions.