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IN VIVO RECOVERY AND HALF-LIFE OF A STEAM-TREATED FACTOR IX (FIX) CONCENTRATE. S.Mörsdorf(1),E.Seifried (2),M.Köhler(1),F.Fasco(1),P.Hellstern(1),G.Pindur(2) Dept.of Clinical Haemostaseology, D-6650 Homburg (1) and Dept.of Haematology, D-7300 Ulm (2), FRG.

The introduction of heat treatment of FVIII or FIX concentrates has reduced the risk of infection, however, has raised the question of a reduced haemostatical effect. Therefore, the in vivo recovery and half-life of a steam-treated FIX concentrate (S-TIM4, Immuno) were investigated in 10 haemophilia B patients from two haemophilia centers. Patients mean age was 33 y (range 17-51 y) and the mean body weight (BW) was 67 kg (range 44-81 kg). Basal FIX levels ranged from 0.007 to 0.03 (median 0,007) U/ml. The patients had not received FIX concentrate at least 7 d prior to the study. Patients 1-4 received 4 different lots, patients 5-10 received one single lot. Blood was drawn before and after 15, 30 min, 1h, 4 h, 8 h, 10 h, 12 h, 24 h and additionally 48 h in patients 1-4. FIX levels were measured using FIX deficient plasma from Immuno (patients 1-10) in center 1, additionally in patients 5-10 using FIX deficient plasma from Merz&Dade. In vivo recovery and half-life were calculated according to Allain (1980, 1984) and given in % and h, respectively.
Results: The table shows the dose and the calculated in vivo recovery and half-life, according to the FIX measurements in center 1 (C1) or center 2 (C2).

Patient		1	2	3	4	5	6	7	8	9	10
dose/kg BW		31	34	30	31	23	28	25	22	20	20
recovery	C1	26	33	36	56	38	54	37	31	52	32
	C2					56	100	60	49	73	67
half-life	C1	23	41	31	30	12	11	8	10	12	9
	C2					23	11	15	14	12	12

Although the apparently longer half-life of patients 1-4 may in part be explained by the longer period of FIX measurements in center 1, the exclusive use of one single lot of FIX concentrate suggests an influence of the lot transfused in these patients. However, laboratory signs of DIC were not present.

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NORMAL IN VIVO KINETICS OF FACTOR VIII (F VIII) AND FACTOR IX (F IX) TREATED WITH TRI (N-BUTYL) PHOSPHATE (TNBP) AND TWEEN 80 FOR INACTIVATION OF VIRUSES. C. Baumgartner (1), B.A. Perret (1), E. Meili (2), M. Furlan (3), H. Friedli (1), J.-J. Morgenthaler (1). Central Laboratory of the Swiss Red Cross Blood Transfusion Service, Bern (1), and the Hemophilia Treatment Centers of Zürich (2), Bern (3), Chur, Basel and Luzern, Switzerland.

Heat treatment has been commonly used for the sterilisation of coagulation factor concentrates. This causes, however, considerable loss of coagulation factor activity; therefore alternative methods have been developed. Two new virus-inactivated coagulation factor preparations were recently introduced by our institution. Their manufacturing procedure includes a lipid solvent extraction step: The cryoprecipitate (F VIII preparation) or the first DEAE eluate (F IX preparation) is incubated with 0.3 % TNBP and 1 % Tween 80 at 24°C for at least 12 hours. (Horowitz, Transfusion 25: 516-522, 1985). Single doses of these preparations (F VIII: median 20.5, range 6-33 U/kg, F IX: median 25, range 9-46 U/kg body weight) were given intravenously to 28 hemophilic patients with minor or no bleeding. F VIII or F IX levels in plasma were determined before and at intervals up to 48 h after injection. The recovery was calculated from the maximum increase of activity and an assumed plasma volume of 41 ml/kg body weight. The plasma half life was calculated according to the procedures described by Morfini (Thromb. Res. 42: I-III, 1986). Results are shown in the table below.

	F VIII		F IX	
	mean ± SD	n	mean ± SD	n
Recovery	83.5 ± 20.1 %	18	53.3 ± 17.4 %	10
Half life	12.5 ± 2.8 h	10	19.4 ± 4.2 h	2

No side effects were recorded. Hemostasis was satisfactory in all patients with bleedings (n = 13). These results are within the range expected for conventional and heat treated F VIII and F IX preparations. We conclude that the lipid solvent extraction procedure as used here does not influence the in vivo kinetics and the function of F VIII and F IX and does not induce any acute toxicity.

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FACTOR IX CONCENTRATES ARE THROMBOGENIC AT HIGH DOSES IN DOGS. J. Ferguson (1), J. Dawes (2), C.V. Prowse (3), P.R. Foster(4), P.A. Feldman (5) and J.K. Smith (5). Wellcome Surgical Institute, Glasgow (1), MRC/SNBTS Blood Components Assay Group, Edinburgh (2), Edinburgh and S.E. Scotland Blood Transfusion Centre (3), Protein Fractionation Centre, Edinburgh (4) and Plasma Fractionation Laboratory, Oxford, UK (5).

A canine model has recently been established to assess the potential thrombogenicity of intravenously infused blood products. Elevated plasma levels of fibrinopeptide A (FpA) were identified as the most sensitive indicator of a thrombogenic response, and this was the only parameter to change significantly when issued batches of factor IX (II + X) concentrate were infused at a dose of 100 iu/kg. After infusion of 200 iu/kg, however, plasma FpA concentrations and FDP titres rose, the APTT was prolonged, and the platelet count and fibrinogen level fell. At this dose, therefore, FIX concentrates which were not identified by in vitro tests as potentially thrombogenic induced a response when infused into dogs.
A batch of FIX concentrate which failed the criteria for in vitro thrombogenicity and was therefore not issued for routine use was infused at 100 iu/kg. Plasma FpA levels rose as did the FDP titre, and fibrinogen concentrations fell, but the APTT was only slightly prolonged. The thrombogenic response to 200 iu/kg of issued FIX concentrate was at least as severe as that following infusion of 100 iu/kg of this rejected batch.
Thus, there is a threshold dose of FIX concentrate above which a severe thrombogenic response can ensue, and the current in vitro tests may not be a reliable indicator of potential thrombogenicity when FIX concentrates are infused at high doses. This should be taken into account when administering unusually high doses, particularly to patients who may have reduced levels of circulating protease inhibitors.

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ALLOANTIBODIES IN HEMOPHILIA B BINDING TO MULTIPLE FACTOR IX (IX) EPITOPES. A. R. Thompson. University of Washington and Puget Sound Blood Center, Seattle, Washington U.S.A.

A high titer, boost responding inhibitor was present in a patient and his nephew, both with severe hemophilia B. On digests of their DNA, Southern blots hybridized to a cDNA were normal. They had no detectable IX antigen, including immunoradiometric assays with a calcium-requiring polyclonal antibody fraction, in either serum or urine (less than 0.03 U/dl). Plasmas from the patient and his nephew had 12 and 25 NIH U/ml inhibitor titers, respectively. They were fractionated over IX-agarose with calcium. Unlike fractionation of rabbit polyclonal antibodies, EDTA eluates did not bind IX. Purified patient inhibitors were eluted at low pH and contained detectable IgG_1, IgG_2 and IgG_4 but not IgG_3, IgA or IgM by radial immunodiffusion. On immunoradiometric assay, each patient's ^{125}I-inhibitor bound to IX on the same solid phase inhibitor, indicating recognition of more than one epitope. Factor IX Ag was readily detected in these assays. The patients' inhibitors competed with binding of ^{125}I-IX binding to each of 3 monoclonal antibodies. Each monoclonal antibody blocked ^{125}I-IX binding to its own, insolubilized species. Similar results were obtained on an inhibitor plasma provided by H. Reisner (Chapel Hill, NC) which, unlike the present cases, contained a calcium-requiring antibody fraction (Briet E, et al Prog Clin Biol Res 150:123-139, 1984). Our patient's inhibitor was tested with two rabbit polyclonal fractions; the calcium-dependent fraction (epitopes in the light chain of IXa) and a specific heavy chain-binding fraction. The latter was from immunoaffinity purification of the non-calcium binding rabbit polyclonal fraction over an insolubilized synthetic peptide containing residues 256 through 269 of the IX sequence. Both fractions, in fluid phase, inhibited binding of ^{125}I-IX to their own but not the other solid phase fraction. The patient's inhibitor did not block ^{125}I-IX binding of the calcium-requiring fraction but did compete with the fraction prepared from the peptide column. These data suggest that the defect in our patient is a mutation between the Gla domain's 2nd or 3rd exons and the second growth factor-like region in the 5th exon.