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PRELIMINARY STUDIES ON THE HEAT TREATMENT OF PFC FVIII CONCENTRATE

1. A description of a Factor FVIII concentrate highly purified and heated in solution has been published by Heimburger, Schwinn, Gratz, Luben, Kump and Herchenhan (Drug Res., 31: 619-622). The procedure involved aluminium hydroxide adsorption of redissolved cryoprecipitate, defibrination by glycine precipitation, precipitation of the FVIII by high sodium chloride concentration, resolution in a sucrose-glycine mixture and heating at 60° for 10h. The FVIII was reprecipitated with sodium chloride, redissolved and dialysed. No details were given of any of the solutions used and the overall yield seems to be 8%, from the cryoprecipitate being 100%.
2. In preliminary studies at the PFC an outdated batch of low activity FVIII concentrate was used. All attempts to heat this material redissolved in its normal reconstitution volume resulted in the rapid formulation of a very firm clot, regardless of any additives used. However if diluted in the presence of some stabilizers no clot was produced on heating. For the final FVIII pasteurisation trial FVIII was redissolved in the given reconstitution volume of pyrogen free water (100ml), this was diluted with 75% sucrose, 0.75M glycine in Ringer's salt solution (200ml). Some samples were adjusted to pH 6.0-6.1 and others to pH 7.0-7.1. These were then heated in a water bath at 60°. Within 2 hours the preparations had become very cloudy although still freely fluid, by 6 hours a flocculate precipitate had begun to form. Results of FVIII assay of samples taken during the experiment were:

<u>SAMPLE</u>	<u>pH</u>	<u>FVIII (u/ml)</u>
Reconstituted FVIII, Immediately frozen	6	3.19
	7	3.33
Reconstituted FVIII, kept at room temp for 6 hours	6	2.78
	7	2.61
Diluted FVIII, Immediately frozen	6	1.03
	7	1.00
Diluted FVIII, kept at room temp for 6 hours	6	1.06
	7	1.05
Sample after 3 hours at 60°, immediately frozen	6	0.55
	7	0.41
Sample after 6 hours at 60°C immediately frozen	6	0.37
	7	0.41

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3. We have been advised that the stabilising preparation used elsewhere for the pasteurisation of plasma proteins including FVIII is 50% sucrose, 2M glycine. This level of glycine precipitates fibrinogen in the PFC product, indeed a similar level is used in the preparation of high purity products. Thus it would seem that the ability to pasteurise a FVIII concentrate is linked to the production of a high purity product.

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