Minutes of FVIII Safety Sub-Committee Meeting held on 15th June 1983 at Headquarters Laboratory.

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1st DRAFT: 16th June 1983
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SUMMARY:

Considerable progress has been made at P.F.C. in producing heat treated FVIII and clinical trials should start towards the end of the summer in Glasgow and Edinburgh. No infectious model for non-A, non-B has been produced yet. The putative 'AIDS' virus must be considered as a potential hazard in FVIII concentrates.

1. Heat Inactivation:

A comprehensive series of time/temperature studies have been carried out at P.F.C. using sorbitol/glycine additive in FVIII and albumin, with albumin and caprylate as control. Different target viruses are used but vaccinia (as suggested by BoB) is most useful as it is (a) DNA virus; (b) double stranded; (c) lipoprotein enveloped; (d) contains a DNA polymerase and (e) can be readily grown and assayed in tissue culture. Individual heat cycles produce differing rates of inactivation at differing time/temperature points when FVIII:C is bioassayed. A preliminary protocol would thus involve (a) 60°C for 10 hours followed by (b) a 30 minute period at 70°C. Under these conditions, log kills for vaccinia, polio 2 and herpes simplex were $>10^8$, $>10^7$ and $>10^7$ respectively. Under the proposed conditions FVIII:C losses are 20-30% but more experiments are needed to confirm these figures. Because the Hoechst additive system (sucrose/glycine) was run in parallel with the P.F.C. additive some internal comparison can be made between the vaccinia killing system and the chimpanzee infectivity reduction ($\cong 10^4$) reported by Hoechst. The inactivation (of vaccinia) does not show the usual residual resistance but instead shows an unusual fall between 12 and 24 hours. This is to be further investigated. Another interesting result is that inactivation of vaccinia proceeds faster in zinc treated FVIII than in albumin.

RIA's of several antigens before/after heating show no evidence of neo-antigens. It is hoped to use matched pairs of heated/unheated products in clinical trials of acceptability in existing adult haemophiliacs at Edinburgh and Glasgow Haemophilia Centres. Dr. Sommerville is also screening for viral antibodies, notably of the herpes group. It is hoped to start clinical trials in Edinburgh with young haemophiliacs later in the year. It is believed that Hoechst have seen one case of post-

transfusion hepatitis with their sucrose/glycine heat treated FVIII and that chimpanzee studies indicate only partial loss of infectivity.

A claim for heat stable (100°C) FVIII from Spain without additives is under investigation. It is believed that the Hyland process includes a 3 day dry heat step at 70°C, but the additives (if any) are unknown.

2. Other (Non-Heat) Treatment:

Following the publication of Nath et al (J. Med. Virol. 10, 1982, p.131-140), it is clear that organic solvents (ethanol, ether) alone can provide significant inactivation of hepatitis B virus DNA polymerase (which is reasonably assumed to correlate with potential infectivity). Since the assumption about the safety of heat treated albumin (60°C, 10 hours) giving $\geq 10^4$ kill may be the additive result of organic solvent and heating, the heat treatment alone in FVIII may reduce infectivity by only say $\leq 10^4$. The question is raised therefore of whether a "belt and braces" approach is required using an additional treatment other than heat. We are of the opinion that this should not be done for the following reasons:

(a) Even a $10^3$-fold reduction in infectivity is worthwhile compared to a non-heated product.

(b) Improved screening and dilution in pools may lower infectivity titres to the point where they are $\leq 10^3$.

(c) A final answer to whether or not heat alone is sufficient would never be obtained with two types of inactivation applied to one product.

(d) Increased losses in FVIII:C will be likely.

Our second choice of an inactivation process is thus either irradiation or organic solvents. To try and define this more closely, a higher purity (porcine "Hyate") concentrate of 10 u/mg was irradiated with 2.5 M rads at +20°C and -196°C. For comparison with earlier results the 1 M rad data are also presented in Table I.

TABLE I

| Product | Temperature | Dose | FVIII:C (% orig) | Solubility | Specific Activity |
|---|---|---|---|---|---|
| "NY443" | +20°C | 1 M Rad | 72% | Fair | ca. 0.5u FVIII:C/mg |
| "Hyate" | +20°C | 2.5 M Rad | 25% | Poor | ca. 10 u " " |
| "Hyate" | -196°C | 2.5 M Rad | 19% | Poor | ca. 10 u " " |

It is clear that even a 20-fold increase in purity does not permit irradiation at 2.5 M Rads nor does cooling in liquid nitrogen help (it was actually worse!). This is rather unfortunate since many commercial (i.e. low cost) irradiation units are set up to operate with a minimum dose of 2.5 M Rads, so reducing this to 1 M Rad is a more expensive option. Some problems have arisen in irradiating our 3 model viruses, so we have only the results for polio 2. With 2.5 M Rads at +20°C, the kill was $\geqslant 10^5$. This could have been $\geqslant 10^8$ but due to freeze/thaw losses in vial during refiltering some 3 logs of infectivity were lost. It is hoped to continue this study with other viruses (Adeno, SV40, Papova) in parallel with heat treatment of the same model and viruses. No significant work has taken place on detergent or monoclonal Ab for HBsAg removal, but Drs. Peutherer and James are hoping to supply larger volumes of better characterised monoclonal Ab's for immobilisation and further evaluation. It is believed that Kabi are about to market a FIX concentrate which has been adsorbed on their capryl hydrazide hydrophobic filter. Kabi have abandoned their own sucrose heat stabilisation method because it was to close to the Behringwerke patent. They were unsuccessful in obtaining a licensing agreement from Behringwerke and are now engaged in licensing a US Government process developed by Purcell some years ago (patent no. ??) based on chloroform treatment. Armour are also negotiating for exclusive North American rights on this same patent. No further details of the

Immuno processes are to hand, but it is likely that the chemical treatment (with diethylpyrocarbonate?) would not be favoured by the FDA because of potential concinogenicity. The 2nd process may be either bile salts or myeloperoxidase. The detergent idea has been claimed in a patent by Shanbron (US Patent, No. 4,314,997) including both hepatitis inactivation and endotoxin removal. However, no supporting data is presented for the former application

3. Acquired Immuno-Deficiency Syndrome (AIDS):

Although not proven to be a virus, this apparently infectious agent has been found in haemophiliacs in the U.K. It would seem wise to try somehow to encompass AIDS inactivation along with HBV and NANB inactivation schemes. In this regard, it reinforces the choice of heat or irradiation as opposed to antibodies, adsorbents or detergents which are likely to be more specific to a particular class of virus. Taking a pessimistic view, some viruses are known with heat resistance up to 80°C, so 70°C may not be sufficient. Some guesses as to the nature of AIDS virus (?) have been excluded (viz. CMV and swine influenza) whereas others have been reinforced by clinical data (viz. Adeno type 43/44 and human papilloma virus). Recent work has shown some association between AIDS and human T-cell leukaemia virus, an (RNA) retrovirus. In view of this, model viruses systems including these viruses are to be set up in tissue culture if possible. SV-40 (double stranded, similar MW to hepatitis B) may also mimic the human papova virus. Because high risk category donors are used as a source of HBsAg, some concern has been expressed about a possible AIDS risk in Merck, Sharpe and Dohme hepatitis B vaccine. This material is prepared by inactivation with pepsin, urea and formaldehyde and the manufacturers claim a $10^5$ kill at each of these 3 steps.

4. Hepatitis B Vaccines and Diagnostic Reagents:

Considerable activity is taking place worldwide in the production of conventional and genetically engineered vaccines against HBsAg and core antigen. The Greater Glasgow Health Board has already purchased ⩾ 1,000 doses of the MSD vaccine. This was to have been used in a long stay home for mentally handicapped children who were seronegative, as well as VD clinic staff, renal unit staff and seronegative haemophiliacs, however the AIDS rumour have effectively stopped the utilisation of this vaccine. Progress in the USA, Japan and Edinburgh has been made on the synthesis of HBsAg polypeptides in E. coli and yeast, the synthesis of whole HBsAg particles in E. coli and yeast and more recently the synthesis of HBcAg in E. coli. One firm (BRL) is marketing a $^{32}P$ labelled DNA probe for HBV DNA. Although not exactly equivalent to an infectivity assay, (and certainly not as sensitive as an infectivity assay) this makes it possible to measure intact viral DNA directly without the need for an animal or tissue culture model. Technical experience of DNA hybridisation technology seems an urgent priority for the Scottish B.T.S. before this assay could be set up. For a useful review of this field see Klausner et al. in Biotechnology, 1, 1983, p.471-478.

5. Non-A, Non-B:

As predicted in our earlier reports, a serological (DS-antigen ELISA) test for NANB has been reported by the Organon Group (Duermeyer et al, J. Med. Virol. 11, 1983, p.11-21). The results were very interesting, however the prognostic potential of the assay remains to be confirmed. A trial to do this is being undertaken by Dr. McClelland in conjunction with Dr. Howard Thomas (Royal Free Hospital, London), who has a similar NANB antigenic marker. The trial is necessarily rather long-term, consisting of placing donor samples in a deep freeze and waiting until a recipient develops NANB. The samples are then analysed for the DS-like antigen retrospectively.

A report has been published (Fields, J. Med. Virol. 11, 1983, p.59-65) claiming that HBV and NANB are unrelated (≤ 5%) by DNA hybridisation studies. Another report from N.I.H. (Lancet, 1, 1983, p.63-64) mentions the transmission of NANB by cryoprecipitate. So far no significant elevation of ALT marker has been obtained in several individual tamerins (2 different species) at P.H.L.S. Colindale. In addition to agent 'H' (from N.I.H.) and the Armour FVIII concentrate (U 70902), Hazel Appleton has also been using Bob Hopkins' putative NANB agent. One animal died, but unfortunately Dr. Appleton was absent from the laboratory and a proper post-mortem and liver biopsy was not performed. This study will continue with new individual animals being injected i.v. (the previous batch were i.m.) with the selection of putative NANB agents. The prospects for success in this area are not very good. No further contact has been had with Major Le Duc (Walter Reed) about owl monkeys nor Inveresk about contract chimpanzee work. In respect of the latter, it seems likely that since IRI were merely acting as agents for a facility in the USA (? Phoenix Primate Colony?) competition with N.I.H./BoB sponsored research is inevitable. In this case, we are likely to have a low priority for scarce animal resources and inevitably the work will be considerably delayed. On the credit side, it seems certain that commercial and/or government organisations in the US will already have planned similar experiments to those proposed by us (e.g. NANB or B viruses in FVIII concentrate and sucrose/glycine and pasteurisation) and that the results of these are likely to be publicly available before we can make significant progress. Whilst this is not a very satisfactory substitute for testing sorbitol/glycine protection, it could give us additional justification for proceeding directly to human subject testing.

6. Proposals:

(a) Further characterisation of heat treated FVIII to assess potential

neo-antigens.

(b) Procurement of marker viruses, growth and assay systems e.g. Adeno, SV40, papova.

(c) Reformulation of above freeze dried viruses in vials for irradiation at 2.5 M Rads.

(d) Heat treatment/protective solutions to be developed for other (?FIX) products.

(e) Continuous re-examination of second options (e.g. solvents).