

SNBTS FVIII STUDY GROUP

SECOND REPORT OF THE SAFETY ACTION GROUP OF MEETING HELD AT SNBTS
HEADQUARTERS UNIT, LIBERTON ON 30th MARCH 1982

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For circulation to all group members prior to next meeting of the group
on Thursday, 3rd June 1982, 9.30 a.m. at Headquarters Unit, Liberton.

SUMMARY

Possible courses of action are becoming clearer, also limitations are very evident. It is proposed that action continue to investigate the effects of heat, radiation and hydrophobic adsorption on FVIII activities. It is also proposed that action be taken to investigate other infectivity assays than chimpanzees, notably owl monkeys, using established colonies. It is also proposed that a model of hepatitis B inactivation be set up using Woodchuck hepatitis virus as the closest analogue. This will require either assay of

samples in the U.S. or establishment of a Woodchuck colony here. Finally, it may also be desirable to write a research grant to apply for sufficient funds to use the existing commercial chimpanzee infectivity model. Efforts are continuing to make personal contact with leading groups in the field (e.g. NIH) to stay abreast of current and future developments.

Apologies for absence were received from Bobby Sommerville.

Roughly speaking, three courses of action are being undertaken simultaneously. These are:

1. Study of the conditions of inactivation by heat, irradiation and hydrophobic adsorption on the various FVIII complex activities.
2. Investigation of possible infectivity assays.
3. Procurement of known positive, titred infective material, namely hepatitis B, non-A, non-B and Woodchuck H.V.

Detailed actions under these headings are as follows:

1. Inactivation Processes:

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- (a) P.F.C. (Alex McLeod, Peter Foster and ?) should continue their work on the heat process as developed by Behringwerke. This consists of heating to 60°C for 10 hours in the presence 50% sucrose and 2 M glycine and has a yield of biological activity of 8%. Attempts must be made to confirm this with existing intermediate purity material, see how the newer higher purity material behaves, and try to improve the yield figure considerably. This latter work could fit in well with existing work of Peter Foster concerning metal ion and poly acid salt additives. We are entitled to assume the results of BoB that 60°C/10 h inactivates non-A, non-B by chimpanzee assay (Tabor et al).
 - (b) DSP will arrange for more samples to be irradiated at different temperatures and from different degrees of fibrinogen removal, to see the effect of these variables. Supplies of porcine high potency FVIII:C may have to be purchased if suitable material is not

procured gratis from Speywood. At present, it seems that low fibrinogen is a pre-requisite for a soluble product following 2.5 mRad, though reduced water content and/or low temperature during irradiation could obviate this problem. Unfortunately, the existing commercial irradiators do not offer low temperature processing.

- (c) DSP will synthesise capryl hydrazide agarose (as per Einarsson's paper from Kabi) and see if it removes the different activities from a FVIII complex. If it does remove them, conditions must be sought to prevent major loss of FVIII:C especially. If it does not remove them (desired result) we are entitled to assume that HBV will be removed. However, this is not a major benefit since HBV is a minor risk now compared to non-A, non-B hepatitis. There is a good chance (which will require proving) that non-A, non-B will be removed also by this approach. If the FVIII:C is recovered in high yield, alternative chemistry for synthesis will be investigated (e.g. epoxide - mercaptan coupling) to give the same unchanged C₈ alkyl hydrophobe, but to avoid the problem of patent piracy. Furthermore, (since it is implied in Einarsson's paper) a better alternative to non-cross linked agarose will be tried. The best candidate on the grounds of chemistry, rigidity and porosity is Sephacryl S-1000.

2. Infectivity Assays:

Because of the time taken to solve this problem, it is appropriate that efforts be made now before any positive results are available from the inactivation study. One general point should be made and that is that since heating (60°C/10 hrs) is now widely held to be effective in destroying infectivity, we might be able to dispense with an infectivity model completely and go straight to human trials (this is what Behringwerke are doing), if we could improve the yield of FVIII:C after heating.

- (a) RS is contacting various persons in North America with a view to arranging infectivity trials of hepatitis B and non-A, non-B in owl

monkeys. Colonies exist in Panama (Gorgas Institute) and in Maryland (Ft. Detrick). Other colonies exist in J.K. and JW is pursuing these for more details. Both Edinburgh University (Bush Estate) and Inveresk Research are able to offer commercial rates of colony care at least an order of magnitude less than chimpanzees and with a much shorter time scale. Unfortunately, on paper at least, the owl monkey is unlikely to be susceptible to human hepatitis virus B (and non-A, non-B). The latter are DNA viruses, belonging to a separate class ("slow viruses") from the hepatitis A virus, which is an RNA virus, similar in many respects to the enteroviruses like polio.

- (b) Information is still being gathered about existing chimpanzee colonies, and how they are used, what results are being obtained and at what costs. It is clear that chimpanzees can be used at a much younger age than was previously mentioned (2 yrs vs. 3 yrs). It is also rumoured that NIH costs (contracted to a colony in U.S.) are lower than those in Liberia. As we are not a commercial concern (?) it might be possible to use this colony instead. No details have come to hand yet about Zuckerman's chimpanzees in London.
- (c) Since our last meeting, some details have surfaced concerning the Woodchuck hepatitis virus (Werner et al, J. Virol., 1979, p.314-322) which is a close homologue of human hepatitis virus B. It has about 5% of DNA homology and is physically identical to the human material. The clinical sequelae are also similar. It is not known (to me) whether this poses an infectivity threat to humans, but I am assuming it is of low risk. In addition to Werner's group in Philadelphia, a second group headed by Dr. Gerin is also using this model in Washington D.C. It is a prime candidate for modelling the physical inactivation processes and we now propose that it replace the earlier proposal of different unrelated viruses. Most importantly, an infectivity assay is available and it can be handled in an ordinary laboratory. This makes it a very good candidate for a research grant submission. I am

presently unaware of any Woodchuck colonies in the U.K., but this could be pursued by DSP or JW.

3. Procurement of Infective Material:

- (a) This has proved a frustrating search. Various people were contacted in the U.K., but none had much to offer. C. Rizza in conjunction with J. Craske are running a prospective trial of "first time" haemophiliacs receiving NHS and commercial concentrates. Samples (10 ml) are taken at regular intervals and Craske uses these for putative screening tests. CR has the liver enzymes done and all data relevant to batch numbers are entered on a computer data base, together with other regular haemophiliacs who report incidents. Depending on who you ask, the infectivity of NHS/Commercial concentrates in first time haemophiliacs (receiving 3,000 units from batches of 5,000 donations) is either 100% (J. Craske) or 50% (C. Rizza). Clearly this is non-A, non-B at low dilution. Hepatitis B is much less common, it is said that it takes 4 exposure-years before this is acquired. Thus, we must assume that all batches of NHS FVIII concentrate and commercial concentrates (of 5,000 donations or more) are positive for non-A, non-B. I doubt that the levels would be detectable however, (even assuming that we had an RIA/IRMA for non-A, non-B) and thus we cannot even guess at what their chimpanzee infectivity titre might be. In an effort to get hold of titred concentrates, I have written/telephoned Drs. Gerety, Fabor and Alter at NIH/BoB, but so far nothing has materialised. They are of the opinion that "factor concentrates" are one type of virus, 'F' and plasma is another type of virus 'H'. I suspect that the situation is more complicated than that, but the conclusion is the same, namely high titre non-A, non-B ($\geq 10^3$ ciu/ml) does not exist in concentrates, so will have to be obtained by plasmapheresis of infected haemophiliacs. Dr. Alter at NIH Blood Bank is alleged to have this material.
- (b) Whilst pursuing the possible "black list" of batch numbers, I was led

to Duncan Thomas at NIBSAC, who did have two library samples of Armour AHF batch J70902, which was reported in November 1980 to have given rise to three separate 'hepatitis' incidents in 3 separate individuals. Unfortunately, the 3 episodes were "B", "non-A, non-B" and "uncharacterised" so either the concentrate is multiply infected or the clinical data are very unreliable. So far this is the only material I have actually got my hands on, but it illustrates the problem of small sample size and the impracticability of doing e.g. chimpanzee infectivity studies on such material, leaving only one vial for experimental work! It was pointed out that most FVIII batches are consumed long before any hepatitis is seen/reported, but in a few favourable cases of larger batches and or smaller Centres, some recall does result in a number of vials being returned. I understand that Jim Smith/Elstree gets this material, though whether it is available to us is another matter! JS alleges it could be salvaged on about one occasion per year.

- (c) This latter information raises the interesting question as to what happens to Scottish material? It is inconceivable that we do not have frequent non-A, non-B contamination. Do any vials survive long enough to be withdrawn? Are the clinical reports reliable? Do "first time" haemophiliacs get special attention as regards LFT's, reporting of symptoms and segregation of batch numbers? It seems entirely likely that PFC's library of FVIII:C concentrates must contain significant quantities of non-A, non-B material, albeit diluted. If we could identify a particular batch with certainty, this would be of some value for future work in this area.