

FVIII SAFETY SUBCOMMITTEE

Report by Secretary to the Committee Meeting of the FVIII Study Group to be held at SNBTS Headquarters Unit, Liberton on February 2nd 1983.

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(No formal meeting of the Subcommittee members has taken place since the previous meeting, but we have been in contact on specific points by telephone)

SUMMARY

No major developments have occurred since our last meeting on Thursday, 14th October 1982. However, a number of small projects are proceeding at the practical level and these are reported in detail here.

Essentially, our earlier predictions and choices are confirmed by the work of others and a useful degree of coherence is appearing worldwide.

1. INACTIVATION

- (a) Heat treatment continues to be the "best choice" and additional evidence is provided (mainly by word of mouth) that manufacturers in Germany, USA and UK are actively working on heat treatment. Patents have been issued to Behringwerke in Germany (No. 2,916,711) and the USA (No. 4,297,344) for the heat-stabilization of factors II, VIII, XIII, ATIII and plasminogen using glycine-sucrose. An additional advantage claimed for this process is low fibrinogen. Not mentioned however is the yield of FVIII:C which is likely to be low. Cutter and Hyland are also pursuing heat treatment, and it is reported that Cutter are patenting other sugars and Hyland have empirically found around 60% yields with one raw material, the reason for this is not known.

A copy of a report from the Bureau of Biologics meeting on 9th September 1982 summarises the views of Dr. Kosow (ANRC) which confirms earlier reports that protein denaturation is the likely route of inactivation by heat.

Heating of NANB has proved effective with 100_{CID} using 60°C/10 hrs.

- (b) Radiation. Bruce Cuthbertson has now supplied titred viruses (Polio 2, Adeno 5 and SV40) in suitable vials. Together with suitable controls these have been frozen, thawed, freeze dried and irradiated with 2.5 M Rads at 20°C (Ethicon). It is hoped to have some log infectivity reduction data at the 2nd February meeting.
- (c) Adsorption. Attempts to adsorb ¹²⁵I-HBsAg tracer from normal plasma failed using both solid phased monoclonal and polyclonal (equine) antisera. The reason is not yet known, but is discussed further below under "Raw Materials".
- (d) Purification. ¹²⁵I-HBsAg tracer was successfully used in a small scale model Zinc precipitation process starting with PFC intermediate purity FVIII:C. At an appropriate zinc concentration only 10-15% of HBsAg was eliminated in the fibrinogen (waste) i.e. 80-90% of HBsAg was retained in the FVIII rich supernatant. On this data alone, one would therefore not expect any reduction in infectivity. However, we have not shown that 22 nm HBsAg and 43 nm Dane particles behave similarly in

-3-

this system.

- (e) Detergents. Considerable extra data on the effect of detergents on HBsAg and Dane particles was obtained from Pat MacKay's thesis (1977). Preliminary experiments in the H.Q. Lab with Triton X-114 (1% v/v) at 37°C for 30 mins (using phase separation) with ^{125}I -HBsAg confirmed her data that the HBsAg 22 nm structure is remarkably resistant to non-ionic detergents.

2. INFECTIVITY

- (a) Marmosets. Dr. Hazel Appleton (PHLS, Colindale) has agreed to inject both *S. labiatus* and *S. oedipus* (one animal each) with NANB agent H as supplied by NIH at 10^{-3} dilution (probably $\approx 10^5_{\text{MID}}$). No positive reports of infection have been obtained so far. However, the BoB were of the opinion that the Marmoset model is unreliable for NANB and only chimpanzees are suitable for this work. In view of this, it rather escalates the need to obtain funding for a chimpanzee study.
- (b) Chimpanzees. Preliminary approaches have been made to IRI to obtain quotes for supply and handling of 2 animals for one year. On the basis of this figure, we should be able to draw up a grant proposal. Several important points relating to the chimpanzee NANB model are made in the BoB report:
- (i) Colony bred animals are more suitable than wild caught ones
 - (ii) Non infected (i.e. successfully inactivated) products must be followed up in each individual with a successful infection with the same untreated product
 - (iii) Titrated inoculum must be used
 - (iv) Suitable inocula and animals are in short supply
 - (v) The carrier product e.g. FVIII:C should be free of IgG to avoid immune neutralisation of virus.
- Most of these points are less serious with HBV studies.
- (c) Cloned HBV DNA. Reports produced by Dr. Cuthbertson, from the Athens meeting on Immunoprophylaxis (held 15-18th November 1982) noted that a group from Heidelberg had prepared cloned HBV DNA and studied its infectivity via different routes in chimpanzees. The results may be summarised as follows:

-4-

Experiment No.	Route	Infection
1	i.v. peripheral	-
2	i.v. hepatic	+
3	intrahepatic cell fusion	+

A second series of experiments confirmed that no infection ensued when 1,000 times more DNA was given by the peripheral i.v. route than had proved infective via the intrahepatic route. This is very important as it confirms our suggestion that "naked" detergent stripped HBV should be considerably less infective than intact Dane particles.

- (d) A strong statement was also made at the Athens meeting that HBS vaccine would make a significant impact on individuals who were at risk. We should bear this in mind when planning long term developments.
- (e) The BoB felt that even if chimpanzee studies were satisfactorily completed, studied in high risk patients, e.g. haemophiliacs, should still be carried out. We might wish to consider this as an alternative to chimpanzee studies.

3. RAW MATERIALS

- (a) We have received sufficient NANB agent 'H' to make up 11 vials of 1 ml each diluted in foetal calf serum plus gelatin + lactose to 10^{-3} dilution i.e. approx. 10^5 MID or 10^2 CID/ml. So far no attempt has been made to procure titred HBV inocula, but it is likely that the same source (NIH) can supply this for the study if we get funded for chimpanzee work.
- (b) Armour Factor VIII (Batch U 70902) has also been sent to Dr. Appleton (PHLS), but this will not be injected unless the agent 'H' source of NANB fails to elevate ALT within 3 months. Assuming the injections started in October, this decision should be made by the end of January.
- (c) Four different monoclonal antibodies against HBsAg were received from Dr. K. James. The pooled antibodies were coupled to solid phase silica (3,000 Å pores). Evaluation of binding to ^{125}I -HBsAg was totally negative. In order to locate the source of failure, we also coupled 5 ml aliquots to Elstree hyperimmune equine anti-HBS (2 horse pool) to Sephacryl S-1000 and to Sepharose 4B. None of these solid phases bound more than 20% of the counts (and the non-specific binding was

-5-

high at 10%), from the specific activity ($22 \mu\text{Ci}/\mu\text{g}$) and the counts used, we calculate that 2 ng/ml of HBsAg was used in a total volume of 5 ml of plasma. Failure to adsorb at this level is not unexpected for monoclonals when solid phased, but is unexpected for polyclonals after solid phasing. Whether coupling failed or whether tracer quality was poor (more likely) must await re-testing of solid phases with new tracer which we hope to get from Dr. Peutherer.

- (d) With a view to screening detergents via a DNA polymerase "unmasking" assay, arrangements have been made with Dr. Peutherer to train one of the H.Q. staff in setting up this assay. Because of the hazardous nature of the materials, this work (if started) will have to be done in the virus isolation laboratory in Dr. Peutherer's laboratory.
- (e) If the solid phase horse or mouse antibodies can be got to work, it should be possible to build a one-tube low-hazard Dane particle DNA polymerase assay as a screening system for detergents. This would mean more work could be done more quickly in low containment laboratories.