To: D. Lewis
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K. Dunbar
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M. Samuelsson

cc: J.D. Michelmore
A. Sheppard
Master
Day
File

From: C.R. Bishop

Date: 4th July 1986.

Re: HTLV III Ab SERO-CONVERSION REVIEW

Although a little late, I enclose for your information a copy of an article from Cutter Laboratories which appeared in the June 14th edition of The Lancet, page 1389, which is virtually a repeat of a letter from the same Company on page 1281 of The Lancet, 31st May.

Both these articles, besides attempting to clarify their own heat-treating procedure, identify ours as being implicated in the cases quoted.

The decision has been taken to respond to The Lancet within the next 2-3 weeks with a carefully prepared "defence" statement setting the facts straight. As soon as this document is prepared, copies will be forwarded to you together with a Technical Bulletin from Robert Christie. However, in the meantime, this subject is sure to be raised again and in order that you are well prepared, I enclose, besides copies of the recent Lancet letters, further copies of Robert Christie's Technical Bulletin and paper relating to the Dutch case, The Lancet Article of March 15th relating to the Chapel Hill case and also the Technical Bulletin and paper prepared by Robert Christie of the 25th March on the McDougal article.

In the Cutter letter of the 14th June they make reference to the McDougal paper. The letter is terribly misleading in that the McDougal article also states that the time required to reduce the titre ten-fold (1 log) at 60°C was 32 minutes in the dry state compared with 24 seconds in the wet. It also states that the virus is undetectable at 60°C for 20 hours as per the information found by the Paul Erhlich Institute and our own Meloy Laboratories but, of course, no mention is made of the efficacy of our heat treatment in the letter. It is rather what is left unsaid in the letter which is causing the confusion.

The defence document referred to above will be based on the following information which please feel free to discuss openly with any of your contacts but under no circumstances let copies be made.

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INTRODUCTION

The suggested implication of Armour heat-treated FACTORATE in HTLV III Ab sero-conversion is based on two cases, both of which received prior treatment with non-heat treated products.

CASE 1 - THE LANCET: APRIL 5TH, 1986, P.803 - W. VAN DEN BERG ET.AL.
(See Technical Bulletin - R.B. Christie - 7.4.86)

Amsterdam

- No records since before January 1984.
- Single male, age 27, with non-married heterosexual relationship living in Amsterdam.
- Received product from AIDS donor.
- No viral growth from patient serum cultures.
- Possible other risk factor unidentified or immune response to inactive virus or viral fragments.

CASE 2 - THE LANCET: MARCH 15TH, 1986, P.612 - G.C. WHITE ET.AL.
CHAPEL HILL

- Known i.v. drug abuser, last located in Mexico City.
- No follow up.
- No viral growth from patient's serum cultures.
- Possible conversion due to i.v. drug abuse.

The 'defence' against FACTORATE should take into consideration the following factors:-

a. In-Vitro

- Many investigators, including J.S. McDougal et.al. - Jnl. Clin. Invest., Vol. 76, August 1985, p.875-877, confirm 60°C at 30 hours in dry state results in undetectable levels of virus.
- This is in line with Meloy Labs. - internal memo 3.12.85 - which shows Armour process to inactivate >5.5. logs of HTLV III.
- Poster no. 387 (p.220 of Abstracts) - A. Werner et.al. - XVII W.F.H. Congress June 1986, also demonstrates undetectable levels at 60 °C for 20 hours dry.
- AIDS donor batches re-checked and no viral growth achieved or Ab. detected in any batch.

b. In-Vivo

- Felding P., Nilsson I.M., Hanson B.G. - The Lancet 1985, ii:832
  No sero-conversion in 46 sero-negative in the short term follow up.

- Preson E. et.al. - The Lancet, July 27th 1985
  No sero-conversion in 2 "clean virgin" treated with Armour FACTORATE H.T. exclusively >12 months ago.

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- Personal communication - Pettigrew A., Royal Infirmary, Glasgow 1986
  No sero-conversion in 1 "clean virgin" treated exclusively with Armour H.T. >12 months ago.

- Personal communication - Smit Sbinga C., Groningen - B.S.H. Congress ENGLAND 1985
  Confirmed at XVII W.F.H. Congress, Milan. No sero-conversion in 15 sero-negatives treated exclusively with Armour H.T. >12 months ago.

- Maggs - University College, London.
  No sero-conversion in 1 "clean virgin" treated with Armour H.T. >12 months ago.

- Kernoff P., The Royal Free Hospital, London
  No reported sero-conversion in 1 "clean virgin" treated with Alpha Profilate H.T. and switched to Armour H.T. >12 months ago.

- Amsterdam
  No other reported cases of sero-conversion in sero-negative patients treated with the same batches as Case 1, even AIDS donor H.T. batches.

- Bradford, Birmingham, Leeds, Hammersmith, Great Ormond Street, St. Georges, London, Liverpool, Royal Infirmary etc.,
  No reports from the above, almost exclusively on Armour, or other U.K. Centres of sero-negative conversions since U.K. introduction of H.T. product in November/December 1984. (Armour U.K. Market share 40-50% 1984/85), including H.T. batches from known AIDS donor.

SUMMARY

a. The in-vitro and in-vivo evidence on both intensively infused and "clean virgin" patients (even on those known to have received AIDS donor material) points to either:-

   i) an immune reponse to inactive virus or viral fragments
   ii) other, as yet unconfirmed, 'risk factors' (one was an i.v. or drug abuser) in the 2 cases quoted in the literature.

In addition, no sero-conversion on any other "clean virgin" not otherwise at risk for AIDS has been reported and live virus has never been isolated from Armour's heat treated FACTORATE.

The above is subject to final ratification and update of personal communications.

C.R. Bishop.

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P.S. There was a meeting held in Newcastle by the Reference Centre AIDS Committee, at which the main topic of conversation was the double standards being applied by the Industry with fully screened product and the NHS who are still supplying unscreened product. Unfortunately, there are rumours going about that Armour are supplying unscreened material and this is probably originating from competitor sources and you must, please, leave everybody in no doubt that all Armour material being sold is sourced from donor material which has been screened and tested for antibodies to HTLV III.

the clinical manifestations". The morphological lesions in the case described here point to the reverse. Our findings suggest that substantial functional reserves exist within the striatal system where considerable morphological damage does not find expression in clinical symptoms. One feels tempted to draw a parallel with Parkinson's disease where a loss of 80–85% of nigrostriatal neurones is necessary to precipitate clinical symptoms.[3] A morphometric analysis on the brain will be published later.

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UNUSUAL SEROLOGICAL PROFILES IN AIDS

SIR,—Dr Dalgleish and colleagues (April 19, p 911) report a patient whose antibody profile by radioimmunoprecipitation (RIP) is interpreted as possibly indicating infection with a "variant" virus serologically distinct from the HTLV-III/LAV prototype. A child with cryptococcal meningitis, severe wasting, and thrombocytopenia had been given a blood transfusion years earlier from a donor who had visited North Africa. The patient's sera reacted by RIP to HTLV-III/LAV envelope proteins only. However, such reactivity is common in patients with AIDS. As many as 50% of AIDS patients lack detectable antibodies to the *gag*-related proteins p24, p55, and p17 in RIP, while maintaining antibodies to *env*-related antigens.[1] In addition, *gag* gene proteins of related viruses are more conserved and thus more immunologically cross-reactive than are exterior glycoproteins, which contain many type-specific antigenic determinants. For example, as has been observed for a recently reported human retrovirus from Africa (HTLV-IV), cross-reactivity on RIP indicative of a distinctly different viral infection is seen primarily to *gag* products p24 and p17 and to *pol* products p64, p53, and p34 rather than to the envelope proteins gp160 and gp120.[2] It is more likely, therefore, that the patient presented had infection with the HTLV-III/LAV prototype rather than a new or related retrovirus.

The ELISA reactivity reported for this patient was consistently negative. Although this could be a result of infection with a virus other than HTLV-III/LAV, it is more probable that it represents a drawback of the ELISA test in certain clinical situations. This patient was receiving intermittent high-dose glucocorticoids.[3] Patients taking immunosuppressive drugs (because of cancer or an organ transplant) may be ELISA negative for weeks or months while reacting positively for HTLV-III/LAV by other, more sensitive confirmatory tests (R. M., unpublished).

We suggest caution in interpreting less common serological profiles as indicating infection with a new or as yet unclassified virus when the results are more easily explained as being consistent with infection with HTLV-III/LAV.

Although HTLV-III/LAV variants do exist in Africa the term "variant" can be confusing. Many isolates of HTLV-III/LAV have been identified and analysed by molecular cloning techniques and, though considered variants by restriction enzyme analysis, they are serologically indistinct from HTLV-III/LAV. Isolates of HTLV-IV do seem to be serologically indistinct from the Simian T-lymphotropic virus type III of African green monkeys ($STLV\text{-}III_{AGM}$). Yet, as previously mentioned, immunological cross-reactivity to HTLV-III/LAV exists, especially to the *gag*-related antigens. This "cross-reactive variant" is a distinct virus and can be evaluated on the basis of the degree of serological cross-reactivity with an HTLV-III/LAV antigen source or with an STLV-III or HTLV-IV antigen source. This divides the African isolates of human T-lymphotropic viruses into at least two groups. If individuals with unusual antibody reactivities were to be screened by immunoblotting or by immunoprecipitation with the above viruses the profiles would fall into one of two categories: those whose antibodies react strongly to the HTLV-III/LAV envelope proteins (gp160, gp120, and gp41) and those whose antibodies react to a higher degree with the envelope proteins of STLV-III or HTLV-IV (gp160/120 and gp32). This protocol would help clarify whether a particular seropositive patient was in fact infected with an HTLV-III/LAV virus or with a related, cross-reactive virus. Since HTLV-IV does not seem to be associated with AIDS, identification of infecting virus may then have future clinical relevance.

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HEAT TREATMENT OF FACTOR VIII CONCENTRATE

SIR,—White et al[1] describe seroconversion to antibody positivity to human immunodeficiency virus (HIV) after the use of factor VIII concentrate heated in a lyophilised state, and van den Berg and colleagues[2] report another such incident. McDougal et al[3] have demonstrated that inactivation of HIV is a function of the matrix in which the virus is contained, the temperature employed, and the duration of time for which that temperature is applied. Inactivation in a liquid matrix is more efficient and swift than that achieved in a lyophilised state. Similarly, heating at a lower temperature or for a shorter duration is less efficient than heating at a higher temperature for longer. Reports indicating transmission of active HIV should therefore indicate in reasonable detail the duration of heat treatment, the temperature applied, and whether the preparation was in a liquid or lyophilised state during such treatment, to allow meaningful conclusions about the safety of heated preparations. After all, heat treatment could be said to have been accomplished if a product is heated to, say, 40°C for 30 min.

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1. White GC, et al: HTLV-III seroconversion associated with heat-treated factor VIII concentrate. *Lancet* 1986; i: 611–12.
2. van den Berg W, ten Cate JW, Breederveld C, Goudsmit J. Seroconversion to HTLV-III in haemophiliacs given heat-treated factor VIII concentrates. *Lancet* 1986; i: 803–04.
3. McDougal JS, Martin LS, Cort SP, Mozen M, Heldebrant CM, Evatt BL. Thermal inactivation of the acquired immunodeficiency syndrome virus, human T lymphotrophic virus III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. *J Clin Invest* 1985; 76: 875–77.

⁂Dr Gilbert C. White II (Durham, North Carolina) and Dr W. van den Berg (Amsterdam) have informed us that heat treatment was, in both cases, at 60°C for 30 h in a lyophilised state.—ED. L.

was rapidly frozen by swirling in alcohol and dry ice. The remainder was shell frozen by swirling in alcohol/dry-ice and lyophilised for 48 h with shelf heating to 27°C (80°F) for the last 24 h. Vials were stoppered under vacuum. The moisture content of samples varied from 0·8 to 1·5%. Vials were heated for different periods of time at 60°C by complete immersion in a water bath.

For assay,[4] vials were rehydrated with sterile distilled water. Frozen samples were rapidly thawed by swirling in a 37°C water bath. Titrations were done in 96-well microtitre plates. To increase the sensitivity of detection of small amounts of residual virus in heated samples macrocultures were set up with 5–10 ml of sample in 50–100 ml cultures. Macrocultures were monitored with weekly tests for reverse transcriptase for 4 weeks.

The virus inactivation resulting from heating alone was surprisingly modest, varying between 0 and 1 $\log_{10}$ at 10 h and between 2 and 4 $\log_{10}$ after 72 h of heating. Lyophilisation alone resulted in an additional 0·5–1 $\log_{10}$ of inactivation. These results are consistent with those reported by Levy et al who found a 2·5 $\log_{10}$ inactivation of their HIV isolate (ARV) with 24 hour heating at 68°C.[6]

Heating in the dry state has only a modest sterilisation effect on hepatitis B virus.[7] Furthermore heated factor VIII products have transmitted non-A, non-B hepatitis to patients.[8,9]

The finding of only modest sterilisation process efficacy for HIV adds to concern about the efficacy of this procedure. It should, however, be stressed that this finding does not mean that dry-heat treated products are unsafe with respect to transmission of AIDS. Indeed three studies have reported absence of anti-HIV seroconversion in recipients of dry-heat treated FVIII preparations.[10–12] Purification and processing steps before lyophilisation can remove or inactivate virus, and lyophilisation alone under commercial conditions probably inactivates more virus than is observed with shell freezing. Furthermore some products are heated above 60°C. Nevertheless, these findings indicate the need for caution in relying on the efficacy of dry-heat sterilisation. Long-term surveillance of recipients of such products for seroconversion to anti-HIV is still required.

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1. McDougal JS, Martin LS, Cort S, Mozen M, Heldbrant CM, Evatt BL. Thermal inactivaton of the acquired immunodeficiency syndrome virus, human T lymphotropic virus-III/lymphadenopathy associated virus, with special reference to antihemophilic factor. *J Clin Invest* 1985; 76: 875–77.
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12. Felding P, Nilsson IM, Hansson BG, et al. Absence of antibodies to LAV/HTLV-III in haemophiliacs treated with heat treated factor VIII concentrate of American origin. *Lancet* 1985; ii: 832–33.

SIR,—Letters by Dr White (March 15, p 611) and Dr van den Berg (April 5, p 803) and their colleagues on seroconversion to human immunodeficiency virus (HIV) antibody positivity after the use of factor VIII concentrate heated in a lyophilised state prompts us to review evidence on the inactivation of HIV when heat treatment is applied to factor VIII or factor IX concentrates in a lyophilised state. McDougal et al[1] have demonstrated that inactivation of HIV is a function of the matrix in which the virus is contained, the temperature used, and the duration for which that temperature is applied. Inactivation in a liquid matrix is more efficient and swift than that achieved in a lyophilised state. Similarly, heating at a lower temperature or for a shorter duration is less efficient than heating at a higher temperature for longer. Anyone recording transmission of active HIV via such products should give details of the duration of heat treatment and the temperature, and they should say if the preparation was in a liquid or lyophilised state during such treatment. After all, heat treatment could be said to have been accomplished if a product is heated to, say, 40°C for 30 min.

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1. McDougal JS, Martin LS, Cort SP, Mozen M, Heldebrant CM, Evatt BL. Thermal inactivation of the acquired immunodeficiency syndrome virus, human T lymphotrophic virus III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. *J Clin Invest* 1985; 76: 875–77.

MEDICAL TREATMENT FOR UNDESCENDED TESTIS

SIR,—Dr de Muinck Keizer-Schrama and colleagues (April 19, p 876) report that only 18% of 271 cryptorchid testes descended completely after 8 weeks of treatment. Having analysed their data we find the success rate to be higher than this. Of the 271 testes treated (fig 1, table II) 15 were "vanishing" testes and should have been excluded from the study. 85 out of the remaining 256 did not require surgery, a descent rate of 33% comparable with that recorded by Illig et al.[1] Furthermore, the conclusion that the lowest success rate was in the youngest patients is incorrect. Using a Fisher's contingency table and the GLIM package with binomial error structure option without standardisation of testicular position to that found in the normal population,[2] we obtained the same statistical inference but a different percentage in group C (6–12 years old). On the other hand, with standardisation of testicular position we arrived at the same percentage but this was not significant. Thus it is not the age of the patient but the position of the testis which is crucial for successful descent. Hormonal evaluation based on LH-RH tests without biopsy correlation is insufficient in recognising the deficiency of the hypothalamo-pituitary-gonadal axis.[3] There is a growing body of evidence that cryptorchidism is due to impaired gonadotropin secretion.[4–6] Because they used inappropriate hormonal and statistical (Student's) tests it is not surprising that the team were unable to detect subtle changes in hypothalamo-pituitary-gonadal axis and so reached incorrect conclusions.

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5. Gendrel D, Job JC, Roger M. Reduced postnatal rise of testosterone in plasma of cryptorchid infants. *Acta Endocrinol (Copenh)* 1978; 89: 372.
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