Cutter (16 IV)

MILES

Re: JB 83-418

TO: Ralph H. Rousell

DATE: August 10, 1983

FROM: Richard S. Schwartz

SUBJECT:

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J. Hjorth
P. DeHart/E. Greene
T. Minaga/M.Budinger

TRIP REPORT

I. June 23, 1983 Discussions at Tropon, Cologne, Germany
II. June 23, 1983 Discussions at Bayer Medical Group, Wuppertal, Germany
III. June 27 to July 1, 1983 World Hemophilia Federation, Stockholm, Sweden
July 2-3, 1983 Hemostasis Subcommittee Meetings, Stockholm, Sweden
July 4-8, 1983 IXth International Congress on Thrombosis and Haemostasis, Stockholm, Sweden

SUMMARY

To no one's surprise, much attention was directed at these meetings to new developments in production of hepatitis-free blood products, and the state of the art of genetic engineering. It is now evident that prolonged heating will not eradicate hepatitis B from AHF if virus spike is added just prior to the heating step. A strain of non-A non-B hepatitis has become available for testing in chimpanzees as well, and is readily inactivated by heat. Two manufacturers, Immuno and Biotest, are using non-heat methods to inactivate both f VIII and IX, and preliminary data appeared very exciting. Their methods were not provided in detail, but may be based on inactivation of the viral envelope using lipid extractable solvents. A number of interesting comments were also brought up during the meetings concerning methodologies for testing hepatitis safety in chimpanzees, e.g., marker viruses for spiking materials.

There were only a few presentations made on recombinant DNA technology, but it was apparent work in this field is moving very fast with two groups, Genentech-Speywood and Hyland Laboratories believed to be in the lead. Genentech has already been able to generate probes to M-RNA based on partial sequencing of the f VIII protein sequence. This will enable them to isolate the f VIII gene in the not-too-distant future.

Several presentations on porcine f VIII were of interest and suggest it will be a useful mode of therapy for inhibitor patients.

There was not much new information on AIDS, but the consensus of the Medical Council of the WFH was to convert hemophiliacs to heated AHF materials as soon as possible if cost permits.

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Dr. Giles in Canada has developed a canine inhibitor hemophiliac dog model which should be of great value in studying bypass agents. Professor Nilsson gave an interesting presentation on the use of IGIV to trat an inhibitor patient.

We were informed by the Kabi group of its decision to assign pastent rights to antithrombin III for Japan and Germany to Hyland Laboratories, with Hyland and Cutter sharing the U.S. market.

Lastly, discussions were held with the staffs at Tropon and Bayer on the status of our clinical studies. A clinical experience of Professor Deck in the use of Polyglobin to treat ITP was of interest.

I. June 23, 1983 Discussions at Tropon
Present: R. Froitzheim, H. Niebel, Mrs. Pfeill, Dr. Neumann, C. Spilles, P. DeHart, M. Boyce, R. SChwartz, J. Wood.

A. AHF-HT: The Tropon staff emphasized the urgent need to begin supplying a heat-treated AHF to meet the requests of the German community. They have made a commitment to supply AHF-HT to the Bonn Institute by October 10. They requested Cutter notify Tropon immediately if it will be unable to meet this deadline. They noted that Tropon may sell products even if not yet licensed by the FDA -- all they need is a license from the German authorities. If the composition of Koate®-HT is similar to the old Koate®, then this may be filed as an amendment to Koate®, even if the manufacturing process differs for the two preparations. If the composition is different, however, they may need a new license for Koate®-HT. They therefore need a statement from Cutter as to the composition of the new product relative to the old. If similar, Tropon will file an amendment with the German authorities. Tropon noted German law precludes selling both products simultaneously. Dr. Spilles requested three bottles from each of our Koate®-HT PR lots for clinical testing. They also requested clinical testing on the 30 ml fill size.

We informed the Tropon staff of our plans to conduct clinical study on a dry-heat process as well as the wet heat, and this would result in some delays in being able to supply AHF-HT to them. They reemphasized the need to expedite this. I also requested they not commence sales in Germany until after we have completed clinical trials in the U.S.

Note: At the time of these discussions, we were unaware that technical improvement might allow for production of a wet-heat AHF. Thus, these comments were meant to apply only to the dry heat process, clinical trials of which would not be completed by the October 10 request.

Lastly, they indicated Professor Scharrer of Frankfurt now wants Coombs tests not saline tests as release criteria for AHF. She requests isoagglutinin titers be 1:32 or less by the Coombs test.

E. Greene for action.

B. CMV-IGIV: Tropon is very interested in initiating clinical trials, probably as treatment of CMV disease, e.g., in renal transplantation. They will accept unlabeled vials and will then relabel at Tropon. In order to label, however, they will need to know the relative potency based on the Paul Ehrlich Institute Reference value, and the potency in comparison with the Biotest CMV hyperimmune globulin. We indicated to them this work was already gong on, and results would be forthcoming shortly.

Dr. Neumann indicated that most of the patients planned for their clinical trials might be children. We then did a rough calculation, assuming an average weight of 45 kg, a single dose of CMV-IGIV of 5 cc/kg, repeated x 3, would require approximately 36 vials, 20 ml per treatment (three courses) course. We therefore agreed to ship 700 20-ml CMV-IGIV vials. This would enable them to treat approximately 20 patients.

Tropon also requested a copy of Drew Winston's abstract (April CMV meeting). They indicated a 50 ml fill size would be more desirable than a 20 ml size, especially for adults. For children, a 20-ml fill size might be acceptable. I replied that subsequent lots would be 50 ml size.

C. IGIV: They requested a copy of results from David Bing's studies.

D. Pseudomonas IGIV: Specific clinical trials have not yet been detailed. There are two major burn centers in Germany and apparently both are already commited to studies of IGIV's. Organization of burn studies may therefore be difficult. We therefore agreed to send only 300 vials of Pseudomonas IGIV until further information was available. Tropon requested data on the titers against the Fisher immunotypes in Ps-IGIV. I also promised to send copies of the Pseudomonas IGIV brochure and several articles on treatment (done).

E. Status of IGIV clinical trials in Germany:

1. Professor Goebel in Dusseldorf has just begun a study of Polyglobin 150 mg/kg monthly in children with acute lymphoblastic leukemia (ALL).

2. Professor Maas in Freiburg has been studying Polyglobin in 1° immunodeficiency. He reports a longer half-life for Polyglobin.

3. Professor Stubner, Hamburg, has observed that IGIV promotes in vitro phagocytosis of yeast. He has begun a study of pulmonary yeast infections in patients to be treated with Polyglobin 500 mg/kg.

4. Professor Urbanek, Freiburg, is interested in the oral application of IGIV for rotaviruses.

5. Studies of IGIV in surgical prophylaxis, candida infection, and burns have not yet commenced, but are in the planning stage.

II. June 23, 1983 Bayer Medical Group, Wuppertal
Present: Professor K. Deck and Dr. F. Schumann.

Discussions centered on IGIV, Pseudomonas IGIV, and Alpha-1 Proteinase Inhibitor.

I reviewed the scope of our clinical trials involving IGIV as well as other products. Dr. Deck was especially interested in the animal data for Pseudomonas IGIV and possible additive effects with antibiotics.

Dr. Schumann will be coordinating clinical trials of Alpha-1 PI in Germany and is very eager to begin these investigations. I told him I will send clinical brochures and preotocols at the earliest data when available.

Lastly, Professor Deck is also an attending physician at a local university hospital and in this role he has had a recent opportunity to use high dose Polyglobin for treatment of a patioent with refractory ITP. The patient did not respond to Polyglobin, but did respond to a subsequent course of Sandoglobin. This suggested to him that there were differences in activity of the two preparations for therapy of ITP.

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III. June 27 - July 8, 1983: World Federation of Hemophilia Congress Hemostasis Subcommittee Meetings, International Congress on Thrombosis and Haemostasis.

A. Antihemophilic Factor

1. Hepatitis-free AHF:
As expected, there was much interest throughout these meetings on new methods for production of hepatitis-free products. Formal presentations were made by representatives from Hyland, Armour, Kabi, and Behring, and Immuno had an interesting display.

a. Hyland Laboratories: (H.S. Kingdon, et al, abstract # 140, WFH meeting). Dr. Kingdon refused to provide specific details on the treatment process for f VIII othger than stating it had been heated for a specific period of time. This process was shown to inactivate several animal marker viruses in vitro including equine encephalitis, pseudorabies, 2 DNA, 2 RNA viruses, and viruses with both "c" and "s." Animal viruses were used since Hyland found that the IgG found naturally in factor VIII concentrates inactivates viruses invitro. In vitro assays of the heated AHF included determination of anticomplementary activity and demonstration that all IgG was 7S. In addition, circulating complement levels in vivo were monitored. These studies were performed because of the concern that the heating process might alter the activity of IgG found in AHF concentrates. No differences were detected before and after heating and all IgG was 7S. AHF-HT was given to dogs at 10x the normal infusion rate without difficulty.

The characteristics of the Hyland AHF-HT product are as follows:
VIII C:AG/VIIIC = 1.3
VIII R:Ag/VIIIC = 3.6
VIII C units/ml = 30
VIII C/AG = 38
TP = 2.0
Fibrinogen = 464 mg/dl = 23%
Fibronectin = 12.3 mg/dl = 62%
Fibrin Split Products - same as non-heated AHF
IgG - 105 mg/dl - all 7S

Chimpanzee studies have been summarized previously, but in short, when AHF was spiked with 30,000 chimp infecitous disease (CID) units, both of two chimpanzees developed hepatitis B. Two chimpanzees treated with AHF spiked with 300 CID prior to the heating step developed hepatitis B, but delayed at 32 and 40 weeks. None of these four animals developed NANB hepatitis, but one of two, when rechallenged with non-heated material, did. Both control chimps treated with non-heated AHF developed NANB hepatitis.

b. Armour Laboratories: The Armour representative also refused to disclose the details of preparation, but did indicate their AHF preparation was heated "not in solution" i.e., dry heat. They also tested the effectiveness of their process in chimpanzees. AHF was spiked with 3000 CID units of AYW subtype hepatitis virus and then heated. There was no change in either time, severity, enzyme changes, development of HBsAg or anti-HBs between animals receiving non-heated and heated material. Although he would not tell me the details, the Armour scientist indicated they had heated their AHF as long and as hard as they could, but saw absolutely no effect on hepatitis B virus. They did note heating prevented the early initial rise of ALT, however, suggesting prevention of NANB hepatitis. In order to study this further, they spiked AHF with 3,000 units of Hutchison strain of NANB hepatitis, obtained from Dr. Purcell. 0/3 animals receiving heated material developed NANB hepatitis, but two animals, when rechallenged with unheated material, did develop hepatitis.

The Armour scientist was quite critical of Hyland's animal studies. He noted that Hyland utilized the ADW subtype of hepatitis B, a strain which reportedly does not produce hepatitis as consistently as the AYW strain, especially when used at low inocula (as done by Hyland). In addition, even when it causes hepatitis in inoculated chimpanzees, the incubation period for ADW hepatitis subtype may be quite variable and unexpectedly prolonged. This subject has been recently reviewed by Tabor et al: Use of and interpretation of results using inocula of hepatitis B virus with known infectivity titers. J Inf Dis 147:531-34, 1983.

c. Behring: (N. Heimburger, abstract # 141, K. Köhler-Vajta, abstract 135, WFH meeting).

The Behring product is heated ten hours at 60°C and albumin is added to their AHF as well. They found that the number of hours to inactivate marker viruses was as follows:

Mumps - two hours, CMV - two hours, rubella - four hours, measles - four hours, herpes simplex - four hours, and polio - eight hours. The current yield was stated to be 50%.

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A number of hemophiliacs have participated in a long-term clinical study with this preparation. Liver function tests and hepatitis serologies were monitored monthly. Follow-up on these patients has been three to six months for 12 patients, seven to 12 months for 14 patients, and 13-24 months for eight patients. All patients have been either newly diagnosed or seronegative at entry into the study. To date, none has developed hepatitis B viral markers, but elevated transaminases have been noted in seven, and one patient has had clinical eviodence of hepatitis. This latter case occurred after halothane anesthesia, a known etiology for chemical hepatitis, and of the seven patients with elevated transaminases, all but one had received other blood products as well. Therefore, although some of these patients may have had non A non B hepatitis, the cause was uncertain.

Chimpanzee studies have also been performed. A comment from the audience was critical that the hepatitis spike was added before the fractionation process. Dr. Heimberger acknowledged this - 10^4-10^5 CID units were added to the bulk, then processed. By the final stage, only 1 ng of hepatitis B virus is left in the concentrate.

d. Immuno: Immunodid not present any data at these meetings. However, at their "booth" was displayed a brochure on "Immuno R & D News" which I have attached, which suggests Immuno has developed a method of inactivation of viruses which is superior to thermal methods of inactivation. I inquired with the Immuno representatives and was told they had developed a form of chemical inactivation which was applicable to both factor VIII and IX, which had already been successfully tested in chimpanzees, and which was vastly superior to any other method currently available. They refused to elaborate on the process, but did hint that some details are provided in the company's annual report. They also suggested they would be glad to show me their facilities and discuss these innovations if I were to come to Vienna to visit them! Mr. Froitzheim wondered if Immuno's method may utilize Tween 80.

e. Biotest: An abstract was to have been presented by Dr. Prince but was cancelled. The abstract (WFH # 139) was printed, however, and indicates Tween 80 + ether provides a potent method of inactivating both hepatitis B and the Hutchinson strain of nonA nonB hepatitis viruses while retaining procogaulant activity of coagulation factors.

f. Kabi: Kabi apparently is still working on processes to inactivate hepatitis virus. Our discussions seemed to indicate they would like to license an existing technology if possible from another manufacturer.

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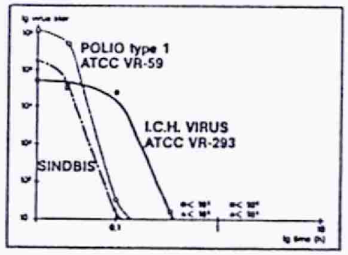
Virus Inactivation in Blood Products ... without Loss of Function

Thermoinactivation of Model Viruses

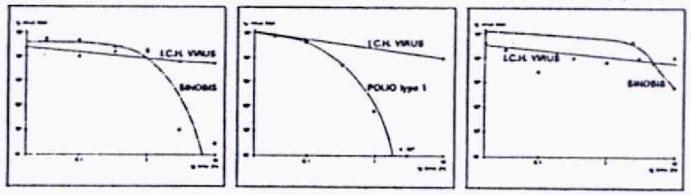
In human albumin (5% solution) at 60° C



First-Generation Methods for Virus Inactivation

Inactivation of model viruses at 60° C
in coagulation factor concentrates stabilized by:

a) high salt concentration | b) sucrose/glycine in solution | c) sorbitol/glycine in lyoph. state

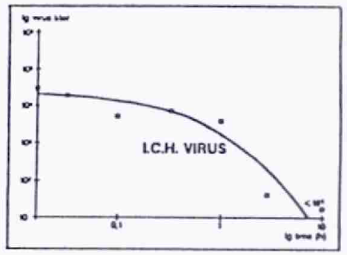


Conclusion:
Stabilizers of plasma proteins also protect viruses against heat treatment

Virus Inactivation in Blood Products without Loss of Function

Advanced Virus Inactivation Methods IMMUNO

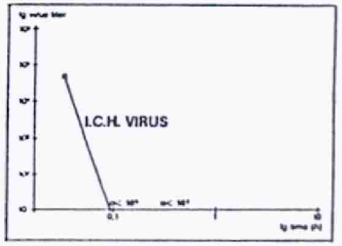
Improved first-generation method to inactivate a model virus in F VIII concentrate



Conclusion:
Inactivation rate is superior to conventional methods, but inferior to inactivation rate in albumin

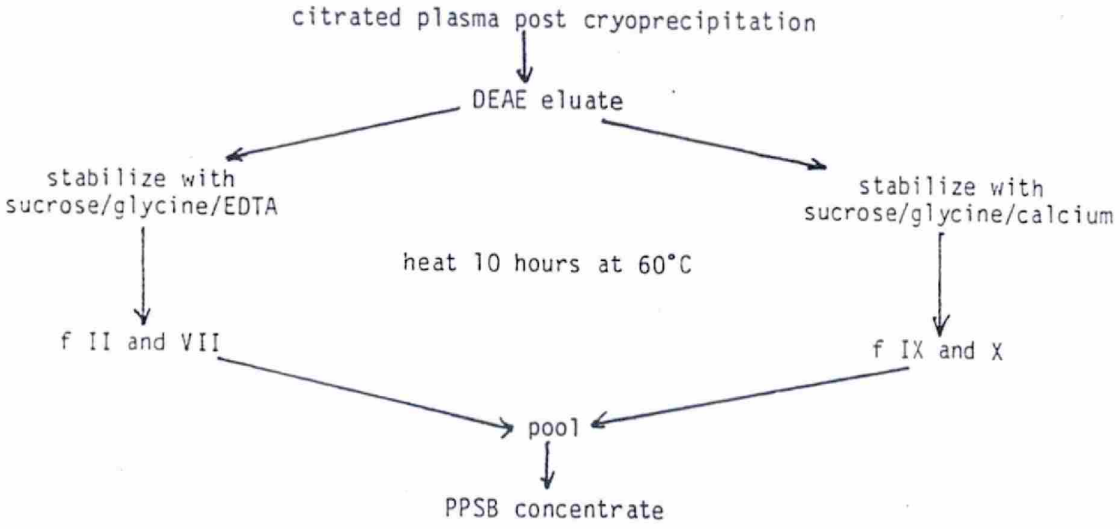
Second-Generation Methods for Virus Inactivation

Fast inactivation of a model virus in F VIII concentrate



Conclusion:
Model virus inactivation rates are superior to inactivation rate in albumin

2. Factor IX - hepatitis safe:
   a. Behringwerke (N. Heimburger et al, Thrombosis and Haemostasis meeting, abstract # 324) They have prepared a hepatitis-safe f IX concentrate based on heat inactivation. An outline of the process is shown below:



The PPSB manufactured in this way had only 0.19% of the HBsAg of the starting material. It was also highly purified, at least five-fold greater purity than currently available f IX concentrates, according to Dr. Heimburger. The specific activity of PPSF was 3.9 (129 mg/500 unit f IX . Both heparin and antithrombin III are also added to the final preparation.

Clinical studies have been conducted in four newly diagnosed childhood hemophiliacs. With followup of three months to one year, none has had evidence of hepatitis or serologic markers while receiving this preparation.

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b. Kabi - (M. Einarsson, etal; WFH meeting abstract # 265) Dr. Einarsson has developed a method to reduce hepatitis B virus in factor IX concentrates using octanohydrazide-sepharose 4B column chromatography. This procedure results in a $\geq 10^4$ fold reduction in hepatitis B virus, but also a 10^2 reduction in non A non B hepatitis virus. In chimpanzee studies, one of two control animals developed non A non B hepatitis, but 0/2 animals receiving treated material did so. This new concentrate has been given to four hemophiliacs, two of whom were newly diagnosed, and none has had abnormal liver function tests, with folow up as long as 12 months.

Dr. Einarsson gave me a copy of her doctoral thesis which summarizes the biochemistry of the process. In discussions with individuals from Kabi, they indicated Kabi may be willing to license this process. Apparently they are already conducting discussions with Hyland Laboratories. I was told if Cutter was interested, it would have to hurry.

3. Methods to Manufacture AHF

a. M. Blomback and colleagues studied the effect of method of freezing of plasma on f VIII recovery (WFH meeting abstract # 10) and concluded fast freezing of plasma is desirable.

b. J. Over et al studied the effect of the cooling rate on f VIII recovery (Thrombosis and Haemostasis meeting abstract # 320). They found that slow freezing of plasma results in the formation of salt and protein gradients which significantly reduce the recovery of f VIII. The optimal condition for freezing of plasma was determined to be 50 minutes at minus 100°C in N_2 gas, resulting in optimal yield of AHF.

B. Genetic Engineering

1. Genentech:

Dr. G. Vehar's lecture entitled "Bioengineering of Blood Derivatives" was a real disappointment, suitable for a Biology 1A course. The only slides of interest listed plasma proteins under consideration and included Alpha-1 Proteinase Inhibitor. The sequence of Alpha-1 PI was shown on another slide, which might suggest Genentech is considering this protein as well.

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2. Speywood-Genentech (Thrombosis and Haemostasis Meeting, abstract # 319).

Using fresh cryoprecipitate as a starting source, factor VIII has been fractionated to high purity using the polyelectrolyte gels perfected by Speywood. In the initial step, using the polyelectrolyte gel, f $VIII_C$ with specific activity of 1,000 U/mg was isolated. This was then further purified using monoclonal antibodies to $VIII_C$ and $VIII_{RAG}$ using affinity chromatography. The main contaminant was fibronectin, which has also been removed using monoclonal antibodies. The final f $VIII_C$ has a specific activity of 4740 units/mg and the overall purification is 331,000 fold, with overall recovery of 19.5%. The predominant band on electrophoresis has a molecular weight of 360K. PMSF, benzamidine, and DFP are used as proteolytic inhibitors during the isolation. If breakdown occurs a band at 210K is found. The Speywood group felt it was important to use fresh cryoprecipitate as a starting source.

In his lecture Dr. Vehar stated it might take five years before f VII by genetic engineering became a reality. In private discussions, however, I learned that Genentech is working on a 14-month timetable, and already has a partial sequence for factor VIII. Although still crude, this apparently is enough to begin preparing probes for the messenger RNA. Some of this work is reported to be in press already. Genentech may also have possibly solved the problem of formation of glycoproteins by using mammalian cell culture instead of bacterial cell systems.

3. Sixma et al (Thrombosis and Haemostasis Meeting, abstract # 321)

Dr. Sixma is working independently but using Hyland Hemofil as starting material passed initially over a sepharose B column, then dextran sulphate sepharose and finally fast protein liquid chromatography. The final purification was >320,000 fold, yield 18%, specific activity >4,500. Benzamidine was used as a proteolytic inhibitor during the procedure. On SDS gel electrophoresis, (both reduced and unreduced), the main peptide bands were at 77K and 80K. However, on Sephadex G200 analytical gel filtration, the native molecular weight was estimated to be 277K.

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4. Kabi-Biogen:

Kabi is working with Biogen on this project, and apparently is still working at the sate of purification. Dr. L. O. Andersson was very impressed by the work of Speywood-Genentech, but commented, as advanced as their work is now, he has heard that Hyland is even further along, having progressed at a very rapid pace with its bioengineering partner, and is probably the leader in the field today.

Dr. Andersson also speculated that ultimately many different molecular weight forms for factor VIII might be produced by genetic engineering, and that many might be clinically useful. This might be important since the lower molecular weight forms, although not native, if biologically active, would be far easier to produce using recombinant technology.

C. AIDS

To date, 21 (possibly 22) cases of AIDS in hemophiliacs have been reported worldwode -- 16 now in the U.S., three in Spain, two in Canada, and ? 1 in Wales. Only one of the U.S. cases is known to be homosexual.

The hemophilic comunity is very much concerned that although the number of cases is still very small, it is being assoicated by the news media with the Gay Community.

There is also much concern that because of widespread fear, both among patients as well as treaters, many patients are receiving inadequate treatment, leading to a dangerous situation for many indviduals. This is feared to be leading to definite harm in some cases. The community therefore perceives the definite need to establish an outlet for communication both for data collection and dissemination of information. Towards this end, Dr. Shelby Dietrich (Los Angeles Orthopaedic Hospital) and Mr. Frank Schnabel have approached the manufacturers of AHF to sponsor an AIDS center at Los Angeles Orthopaedic, as detailed in separate memoranda.

The Medical Research Advisory Committee of the WFH also recommended 1) more research, 2) treatment as necessary, and don't withhold treatment, 3) If cost is not a factor, switch to hepatitis-free products, recognizing there are no data to support this stand. Harold Roberts of Chapel Hill was apparently most vocal, noting that AIDS may be caused by a retrovirus, and such viruses are known to be heat labile. There was apparently some dissent on this last recommendation, but this represented the opinion of the majority of the council.

There were also many discussions on studies of lymphocyte populations in hemophiliacs receiving AHF concentrates, both from U.S. and manufactured in a given country, and cryoprecipitate. Although most found decreased helper/suppressor ratios, some found this was due to a diminished number of helper cells while others reported an increased number of suppressor cells.

D. Hepatitis B Vaccine

Two presentations were made on the results of vaccination of hemophiliacs.

Ulla Hedner (Sweden, WFH abstract # 207) demonstrated that hepatitis B vaccine was immunogenic in hemophiliacs. C. Ganzegel et al (WFH abstract # 208) immunized 34 hemophiliacs with a vaccine prepared in France in combination with hyperimmune anti-HBs plasma. In 16 patients followed for more than a year none has developed hepatitis B.

E. Treatment of Inhibitor Patients

1. Autoplex vs. Proplex - J. Lusher, (WFH meeting abstract # 50) Hemophiliacs with acute hemarthroses were randomized to receive a single dose of either Proplex 75 f IX U/kg, Autoplex 50, or 75 U/kg. Assessment was made at six hours following treatment. The results are as follows:

	Autoplex 75 U/kg	Autoplex 50 U/kg	Proplex
Symptomatic improvement	53.8	51.9	56
Improved joint mobility	51.9	47.8	42.9
Overall effectiveness	51.7	55.6	50

Perceived need for 2nd treatment -- all about 50%

Thus, there were no significant differences in response rates between any of the arms of the study. Hyland is now planning to repeat this study, using a second dose of each preparation if indicated and extending the period of observation beyond six hours.

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2. Use of phospholipids to overcome inhibitors. T. Barrowcliffe (WFH abstract # 52 and Thrombosis and Haemostasis meeting abstract # 802). Dr. Barrowcliffe has previously reported that in vitro, phospholipid protects f VIII from antibody attack and neutralization. He has now tested several commercial and reagent sources of phospholipids in his test systems including Intralipid® (Kabi). Intralipid® did not protect f VIII from neutralization whereas some research reagents did. Further study revealed that phosphotidyl serine (PS) was the most active component for protection with phosphatidic acid being less active. Phosphatidyl-choline (PC), -ethanolamine, and -inositol were inactive. Of interest, therefore, was the finding that Intralipid® contains no PS at all, while the most active reagent in this series had 21% PS. Dr. Barrowcliffe has found one needs 10-20% PS in a reagent to see a protective effect. He has applied for a patent for the combination of PL-f VIII and clinical trials in dogs have already begun. Human studies will be forthcoming shortly.

3. Treatment of inhibitor patients with IGIV. I.M. Nilsson (WFH abstract # 51). A patient with hemophilia B and a 20 year history of an inhibitor was treated with extracorporeal plasma adsorption, immunosuppression, and high doses of intravenous gammaglobulin (Kabi). The inhibitor titer sems to have been suppressed by the IGIV. The details of this patient's clinical course have recently been published (Scan J Haematol 30:458-64, 1983) and the abstract is attached.

   Professor Bloom (Wales) has recently treated a hemophiliac with ITP with IGIV. The ITP remitted, but the inhibitor remained unchanged. Dr. Peter Levine (Worcester, MA) also recently treated a hemophilia B patient with an inhibitor with high doses of Gamimune®, without change in the inhibitor.

   Note: We are aware of one other patient with an adcquired inhibitor who was treated with Gamimune® and immunosuppressive therapy with disappearance of the antibody. Thus, the two patients who responded to IGIV both received concomitant immunosuppression with the IGIV. Perhaps this combination is required to see an effect.

4. Canine model of hemophilia with inhibitor antibodies. A. Giles (Canada) (Thrombosis and Haemostasis meeting abstract # 1081). A hemophilic male miniature Schnauger (MS) was bred with a normal female Brittany Spaniel (BS) and a carrier pure bred MS female. Of interest, five hemophilic dogs from the crossbreeding of BS x MS have, after treatment with canine cryoprecipitate, have developed inhibitors to f VIII, but none of the dogs from the other breeding arms have done so. These inhibitor antibodies cross react with human f VIII, but not porcine f VIII (P f VIII). P-f VIII is effective in stopping bleeding in the inhibitor dogs. These studies suggest inhibitor formation in hemohilia is genetically controlled. This dog model will be extremely useful for study of bypass materials.

5. Porcine AHF (WFH abstract # 159, 160, 161). The overall clinical experience with P-AHF has been favorable with most patiehts with ≤ 30 Bethesda "porcine" units of crossreacting antibody responding. The recommended clinical dosage is 50-100 U/kg for severe bleeds, and 25-50 U/kg for minor hemorrhage. Professor Mannucci uses a formula, based on the inhibitor titer, to determine the neutralization dose of P-AHF required. The incidence of adverse reactions has been quite low - only 0.6% of infusions led to severe reactions. Most adverse ractions occur on first or second treatments; if well tolerated at these times, subsequent reactions are rare. A few patients in the U.K. are actually receiving P-AHF now on a hjome care prophylactic program, and some individuals have been repeatedly treated.

   Although platelet aggregation and thrombocytopenia were probl early in the product's development, with higher degrees of ems purification, this has not been a clinical problem.

   Porcine-AHF is prepared using a polyelectrolyte gel and resul material has a very high specific activity, 25 U/mg. Speywootant now using this same technology to prepare human f VIII of verd is high specific activity for clinical use. y

F. Antithrombin III

1. Büller and Ten Cate administered high doses of AT III via continuous infusion to patients with liver disease undergoing peritoneovenous shunt operations (Thrombosis and Haemostasis abstract # 127). DIC was not prevented.

2. G. Potron et al treated infants with respiratory distress syndrome with AT III, but saw no improvement in survival (Thrombosis and Haemostasis abstract # 128). The dose of AT used in this study was totally inadequate, however, as it faiII I to elevate the AT III of the treated group above that of the led control. These results are therefore inconclusive.

3. Peters and Ten Cate studied AT III levels in neonates with respiratory distress (Thrombosis and Haemostasis abstract # l and found that infants with RDS had significantly lower level3l) AT III than those without and the AT III level correlated wits of survival. h

4. M. Wickherhauser published a single step method for isolation AT III (Thrombosis and Haemostasis abstract # 353). of

5. Ten Cate (Thrombosis and Haemostasis meeting, abstract # 362) studied metabolism of radiolabelled AT III in liver disease a found a mean half-life of 57 hours, suggesting the low levelsnd AT III found in liver disease are due to diminished synthesis of rather than consumption.

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6. Müller-Berghaus et al studied AT III in an experimental animal model system of disseminated intravascular coagulation (Thrombosis and Haemostasis # 1024). AT III alone was ineffective in preventing endotoxin induced DIC where heparin by itself was partially effective. The combination of AT III and heparin, however, was most effective. Surprisingly, AT III levels did not fall in any of their DIC induced groups.

7. D. C. Triantaphyllopoulos presented contradictory data (Thrombosis and Haemostasis abstract # 1126). He found that AT III by itself prevented the death of rabbits induced by endotoxin.

8. Discussions with Kabi group.
Present: L. D. Andersson, R. Hagberg, M. Mikaelsson, Lisbet Javelin (Kabi), and M. Fournel, R. Jordan, R. Schwartz (Cutter).

   Discussions centered around AT III. Kabi informed us patent rights to their process for purification of AT III had been assigned to Hyland Laboratories for Japan (exclusive), Germany, and the U.S., with Cutter also being assigned non-exclusive rights limited to the U.S. and Canada. Kabi indicated rights to Japan and Germany had been offered to Cutter initially, but an agreement was never reached. Kabi threfore proceeded to offer these rights to Hyland, who apparently responded rapidly to Kabi's offer. Hyland will license the entire process of manufacture from Kabi.

   Kabi has recently received its license for At III in Sweden, and apparently sales are much better than they had predicted. The primary use has been treatment of disseminated intravascular coagulation.

   Mrs. Javelin has been supervising Kabi's AT III clinical trials. She will be sending me some materials on their current status. Some of the more interesting applications, e.g., AT III for therapy of neonates with respiratory distrress syndrome, are just getting underway.

H. Protein C

There were many presentations on this subject suggesting increasing interest.

A group in Leiden (Thrombosis and Haemostasis abstract # 1096) estimated the incidence of isolated protein C deficiency to be 1:16,000 and 1% of patients with venous thromboembolism were deficient in protein C.

I. IGIV treatment of ITP

1. C. Wenske et al treated four pregnant women who had ITP and were near delivery with high doses of Sandoglobin (Thrombosis and Haemostasis abstract # 984). Two of the four had excellent responses and the other two showed partial responses, permitting delivery of the babies.

2. Discussions with Kabi

   Members of Kabi told me experience with their own IGIV has been highly favorable, with nearly all patients responding, some with very prolonged responses.

3. Professor Deck of Bayer informed us of a patient in Germany who did not respond to Polyglobin but did to Sandoglobin.

RSS:jb

GRO-C

111 6714

10 IMPROVEMENT OF PLASMA QUALITY AS RAW MATERIAL FOR F VIII:C CONCENTRATES. FREEZING OF PLASMA.

G. Carlebjörk, M. Blombäck, Dept of Blood Coagulation Disorders, Karolinska Hospital, Stockholm, Sweden.

Aim. The influence of different freezing charachteristics on the quality of plasma for Factor VIII:C production has been investigated.
Method. Temperature curves were recorded with thermo-couples when freezing plasma with different equipment. Plasma ampoules of two sizes were used. A plasma pool was divided into three 750 ml plasma ampoules and frozen in a -25°C freezebox, a -80°C freezebox and a -40°C circulated ethanol bath. Each ampoule was individually thawn and processed to cryoprecipitate. F VIII:C, F VIIIR:Ag and fibrinogen were analyzed with conventional methods.
Results. The temperature curves in plasma decreased with different speed down to the freezing-point (with a temperature-plateau of variable length) and proceeded with a second decrease down to the final temperature. Duration at freezing point increased with increasing thickness of the plasma layer. Freezing in rectangular 750 ml ampoules resulted in steeper temperature gradients and shorter duration at the freezing point than for the cylindric 1500 ml ampoules. Ethanol bath at -40°C was superior to both -25°C and -80°C deep freezers in rate of freezing but only if the bath was equiped with circulation and enough compressor capacity to maintain its temperature. Total freezing times for plasma in 750 ml ampoules to -25°C were 10 h, 2 h 30 min, and 40 min with -25°C freezebox, -80°C freezebox and -40°C ethanol bath. The F VIII:C recovery in frozen plasma increased with faster rate of freezing. The purity of cryoprecipitate expressed as F VIII:C/mg fibrinogen as well as the F VIII:C cryo recovery increased with faster rate of freezing.
Conclusion. It is concluded that fast freezing of plasma results in higher F VIII yield and purity of cryoprecipitates from such plasma. Fast freezing of plasma with short duration at the freezing point plateau can preferably be achieved with flat 750 ml ampoules and -40°C bath with effective cooling and circulation.

51 SUPPRESSION OF SECONDARY ANTIBODY RESPONSE BY INTRAVENOUS IMMUNOGLOBULIN AND DEVELOPMENT OF TOLERANCE IN A PATIENT WITH HAEMOPHILIA B AND ANTIBODIES

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A new technique for removal of high titre antibodies in plasma by adsorption to protein A-Sepharose was earlier described by Nilsson et al in a haemophiliac. Recently the same procedure was performed including conventional substitution therapy in combination with immunosuppression (cyclophosphamide). I.v. immunoglobulin was also given in doses recommended by Imbach et al and Fehr et al for treatment of idiopathic thrombocytopenic purpura.

Within a week of the first treatment the patient developed a 15-fold increase in the antibody titre. Following the second treatment, antibodies in low titre could be detected within an observation period of 12 weeks. No evidence was found that the effect on the secondary antibody response was due either to nonspecific suppression of the immune and reticuloendothelial systems or to acting of interfering antibodies. It seems as antibody synthesis was suppressed by the i.v. immunoglobulin.

Later, the patient has been treated twice with i.v. immunoglobulin in combination with factor IX and twice with factor IX alone. Within observation periods between 9 to 4 weeks only traces of antibody has been detected.

The results suggests a new approach to the treatment of haemophiliacs with antibodies of the high-responding type.

52 OVERCOMING FACTOR VIII INHIBITORS: A POSSIBLE NEW APPROACH

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Treatment of haemophiliacs with antibodies to factor VIII is still a major clinical problem, neither high-dose factor VIII not factor IX concentrates being completely effective. An alternative approach was suggested by the finding that factor VIII, when complexed with phospholipid and factor IXa, was protected from antibody attack, and that the major protective effect was provided by the phospholipid.

The protective effects of various phospholipids were assessed by addition to factor VIII concentrates, incubation with human antibodies to VIII:C, and measurement of residual clotting activity in three different assay systems. Six commercial phospholipids used as coagulation reagents were all partially effective, but the most active material was a home-made extract of bovine brain, prepared by a modified Folch method. Of three commercial lipid preparations manufactured for intravenous injection, one was inactive, and the other two were only active at high concentration.

Studies with purified phospholipids showed that phosphatidyl serine (PS) was the most active fraction. Phospholipid analyses showed that the three intravenous lipid preparations all contained less than 10% PS, whereas the bovine brain extract contained 20-25%.

These studies show that factor VIII can be protected from antibody attack by addition of phospholipid extracts which are rich in PS, and suggest that a factor VIII product containing a suitable phospholipid could be of benefit to haemophiliacs with inhibitors.

0127

ANTITHROMBIN III INFUSION IN PATIENTS UNDERGOING PERITONEOVENOUS SHUNT OPERATION: FAILURE IN THE PREVENTION OF DISSEMINATED INTRAVASCULAR COAGULATION.

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Seven patients with chronic liver disease and acquired antithrombin III (AT-III) deficiency undergoing peritoneovenous (LeVeen) shunting for ascites, resistant to medical therapy, were studied prospectively for the development of disseminated intravascular coagulation (D.I.C.). In five of the seven patients selective correction of the plasma AT-III activity to normal was accomplished by continuous infusion of purified human AT-III concentrate beginning one day prior to surgery and continuing five to seven days postoperatively. This rigorous transfusion scheme of AT-III concentrate could not prevent D.I.C. and bleeding. Extensive studies of coagulation and fibrinolysis factors reveal that primary fibrinolysis may play a role in LeVeen shunt associated coagulopathy.

111 6715

0131

LOW CORD BLOOD ANTITHROMBIN III - LEVELS IN NEONATES WITH IDIOPATHIC RESPIRATORY DISTRESS SYNDROME, PREDICTIVE OF A FETAL OUTCOME.

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Division of Hemostasis and Thrombosis and Division of Neonatology, University Hospital "Wilhelmina Gasthuis", Amsterdam and Gaubius Institute, Leiden, The Netherlands.

In a prospective study in 82 consecutive premature newborns (24 healthy prematures (controls) and 58 neonates with IRDS) we investigated the possible significance of hemostatic abnormalities in IRDS. In neonates with IRDS all coagulation parameters were significantly lower than control values and remained so during the first days of life. The mean cord blood AT-III-levels in neonates who developed IRDS was significantly lower as compared to premature neonates who remained healthy (0.19 U/ml and 0.37 U/ml respectively $p < 0.001$). Antithrombin III was the only coagulation parameter which distinquished between neonates with IRDS who survived (mean AT-III level: 0.21 U/ml) and who did not survive (mean AT-III level 0.16 U/ml) ($p < 0.01$). Within the first 6 hours of life an AT-III below 0.15 U/ml was present in 8 patients who developed IRDS, 7 of those died within 48 hrs. after delivery. Autopsy of these neonates showed widespread fibrin deposition and hemorrhage in vital organs consistent with intravascular coagulation. These findings indicate that very low AT-III plasma levels are associated with Disseminated Intravascular Coagulation in neonates with and are predictive of a bad outcome.

39 INACTIVATION OF HEPATITIS B AND NON-A, NON-B VIRUSES IN LABILE BLOOD DERIVATIVES

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Aim
Proposed virus inactivation steps were evaluated for their effect on the infectivity of hepatitis B (HBV) and non-A, non-B (NANB) viruses and on the activity present in coagulation factor concentrates.

Methods
Naturally and artificially contaminated plasma and plasma derivatives were treated with either beta-propiolactone (BPL) + ultraviolet (UV) irradiation, Tween 80 + BPL/UV, or with Tween 80 + ether. Untreated and treated materials were then inoculated intravenously into pairs of chimpanzees which were followed for at least 6 months with weekly determinations of transaminases and HBV serologic markers, and biweekly biopsies for light and electron microscopic study. Coagulation factors were measured by single stage partial thromboplastin times and by immunoelectrophoresis.

Results
BPL/UV treatment inactivated a tubule forming NANB virus in a pooled starting plasma. BPL/UV + Tween inactivated 6.9 log chimp infectious doses (CID-50) of HBV as well as a tubule forming NANB virus contaminating a pooled cryoprecipitate. Tween 80 + ether treatment inactivated at least 6 log CID-50 of HBV and 4 log CID-50 of Hutchinson strain NANB virus. Chimpanzees which received treated material were shown to be susceptible to HBV and NANB viruses by challenge with untreated inocula. Tween 80 + ether treatment was evaluated to determine its effect on coagulation factor concentrates. In AHF concentrates, 70% of procoagulant activity, 93% of AHF related antigen, and all of the ristocetin cofactor activity was retained. AHF procoagulant activity remained associated with von Willebrand factor as judged by gel exclusion chromatography. Analysis of the fibronectin (FN) present in the concentrates showed that FN antigen was fully retained and FN opsonic activity was 72% retained. In factor IX concentrates, no change in F IX coagulant activity was observed.

Conclusions
The above procedures offer practical means for reducing the risk of hepatitis transmission by labile blood derivatives.

135 VIRAL SEROMARKERS AND AMINOTRANSFERASES IN BOYS WITH HEMOPHILIA A - A FOLLOW UP STUDY

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Aim

The purpose of this study was to investigate the effects of F.VIII-concentrate therapy on incidence of hepatitis infection in hemophiliacs.

Patients and Methods

In a follow up of the last ten years we studied more than 100 patients. Since 1980 18 of them were treated by heat-sterilized F.VIII-concentrates either in the case of newly diagnosed hemophilia or in the case of seronegativity as to hepatitis B-virus (HBV). Seromarkers for HBV, hepatitis A-virus, Ebstein-Barr-virus (EBV) and cytomegalovirus (CMV) as well as aminotransferases were determined at irregular intervals. Booster-effects were correlated to the therapeutic regimes. Clinically manifested hepatitis was confirmed in most cases by laboratory data.

Results and Conclusions

The patients treated by sterilized F.VIII-concentrate developed neither clinically manifested hepatitis nor seropositivity for HBV or signs for an infection caused by the other viruses. However, in a 1/4 of these patients a moderate increase of aminotransferases was observed. On the other hand in the conventionally treated group, more then 90% showed seropositivity for HBV-markers, 1/3 of them suffered from icteric hepatitis, and a few patients showed active infection by EBV or CMV (one patient). The results suggest that heat treatment of F.VIII-concentrate prevents transmission of HBV-infection, whereas transmission of NANB-virusinfection cannot be excluded.

140 HEPATITIS RISK REDUCTION IN HEMOPHILIA: A HEATED FACTOR VIII PREPARATION. G.Polana, D Tse, W Thomas, H.S Kingdon, Hyland Therapeutic Division, Travenol Laboratories, Inc. Glendale, CA

A new process has been developed for preparing therapeutic factor VIII concentrate which includes a heating step designed to reduce risk of hepatitis when the product is infused into patients with hemophilia A. Extensive experiments with easily grown "marker" viruses established conditions under which significant kill of hepatitis virus might be expected. Extensive characterization of the final product in comparison to unheated product defined those conditions under which viral kill might be expected, with minimal adverse effects on the product. Animal toxicology and pharmacology studies indicated safety of the heated product. Using the final set of conditions, heated product was compared with unheated product in 12 multiply-transfused patients with hemophilia A. In vivo recovery and half-life demontrated no statistically significant differences between heated and unheated product.

Half-life of heated and unheated factor VIII

	no.of patients	median half-life unheated	hours heated
Study A (Europe)	6	10.2	9.3
Study B (USA)	6	8.0	8.2

There were no adverse reactions, indicating safety and efficacy for the heated product in treatment of hemophilia A Finally, chimpanzees were inoculated with a therapeutic dose of factor VIII concentrate containing both hepatitis B virus (an amount that would be capable of causing hepatitis B infection in humans) and a source of at least one type of non-A, non-B hepatitis. The control(non-heated product) animal contracted both non-A, non-B hepatitis, and hepatitis B, at 5 weeks and 22 weeks, respectively. Two animals receiving heated product developed no hepatitis in 8 months of observation. It thus appears that this new process yields a therapeutic factor VIII preparation which is safe and effective for treatment of hemophilia A, while carrying a reduced risk for transmitting hepatitis when compared with products heretofore available.

111 6716

141 FACTOR VIII CONCENTRATE, HEPATITIS-SAFE: PREPARATION AND CLINICAL EXPERIENCE

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The process for the production of a highly purified and pasteurized factor VIII concentrate is described and the clinical data of the long term observation of patients treated with this concentrate are summarized.

For the production of the concentrate, cryoprecipitate of pooled citrated plasma from donors in Western Europe and USA collected according to the local legal regulations is absorbed with aluminium hydroxide gel to eliminate the factors of the prothrombin complex. Fibrinogen is removed by glycine fractionation and afterwards F VIII is precipitated with sodium chloride. For pasteurization the sodium chloride precipitate is dissolved in a saccharose/glycine solution and heated at 60°C for 10 hours. From this solution F VIII is separated again with sodium chloride. After dissolution the F VIII concentrate is dialysed and sterilized by filtration. Even after addition of albumin for stabilization it contains about 6 units F VIII clotting activity per mg protein, and the ratio of F VIIIR:Ag/ F VIII:C is 3. The product is very pure and does neither contain functional clotting proteins except F VIII nor γ-globulins. The efficacy of the pasteurization step was tested with a solution deliberately contaminated with infectious hepatitis B virus by application to chimpanzees. After pasteurization the concentrate did no longer transmit hepatitis to chimpanzees.

The clinical follow up of this concentrate over several years also showed that it did not lead to hepatitis B infections of patients.

159 POLYELECTROLYTE FRACTIONATED PORCINE FACTOR VIII:C - A REVIEW OF THE TECHNOLOGY AND INTERNATIONAL CLINICAL EXPERIENCE.

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A unique concentrate of porcine factor VIII:C prepared on a commercial scale by polyelectrolyte fractionation has now been in clinical use for 3 years.

Cryoprecipitate from porcine plasma is applied to a column of protonated polyelectrolyte which selectively adsorbs factor VIII:C. The FVIIIR:Ag and associated Platelet Aggregating Factor (PAF) are washed from the column along with other plasma proteins. FVIII:C is eluted in high ionic strength buffer, concentrated, sterile filtered and freeze dried.

The final concentrate is highly soluble, has a purity of about 25 u. FVIII:C per mg. of protein and contains low levels of PAF.

This concentrate has been successfully and extensively used in Europe and the U.S.A. for the treatment of haemophilia A patients with inhibitors to human FVIII.

Bleeding episodes treated have ranged from moderately severe to potentially life or limb-threatening haemorrhages. A number of surgical procedures have been carried out under cover of the material.

Dosages used have ranged from 10 u/kg to over 600 u/kg. Duration of therapy has varied from a single dose to 27 days of continuous treatment. Many patients have received multiple courses without loss of efficacy of treatment or increased incidence of side effects.

Conclusion

Porcine factor VIII:C prepared by this methodology is an effective agent for the treatment of bleeding episodes in many haemophilia A inhibitor patients.

160 DOSE-RESPONSE RELATIONSHIPS AND IMMUNOGENICITY OF PORCINE FACTOR VIII

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A polyelectrolyte-fractioned porcine factor VIII concentrate (porcine FVIII) has been given to 13 "high responder" hemophiliacs with antibodies (Ab) to FVIII coagulant activity (FVIII:C) who had 14 limb-threatening hemorrhages and 3 multiple dental extractions (17 courses,36 infusions). Before the first courses of treatment anti-porcine FVIII:C Ab was always lower than anti-human FVIII:C Ab (mean:4U/ml, range 0-13; versus 16 U/ml,range 3-54),with a mean cross-reactivity of 32% (range 0-74).The dose of porcine FVIII needed to neutralize the Ab was calculated on the basis of plasma volume, Ab titer and the presumed extravascular Ab pool;an additional dose was then given to increase plasma FVIII:C to the level needed to control each bleeding.The mean rise in FVIII:C was 1.22 U/dl/unit infused/Kg body weight (range 0.35-4.74).Clinical responses were proportional to the FVIII:C levels attained in plasma;no patient developed thrombocytopenia; 4 mild pyrogenic-type reactions were encountered; 1 patient had a severe anaphylactic reaction.An anamnestic rise in anti-porcine FVIII:C Ab (3 x the baseline titer) was seen after 7 of the 17 treatment courses;a rise in antihuman FVIII:C Ab was seen in 3 instances only;mean cross-reactivity increased from 32%(range 0-74) before therapy to 46% (range 21-75) after therapy. Porcine FVIII was also used during and after a major obstetrical procedure in a woman with an Ab acquired post-partum (43 U/ml anti-human and 3 U/ml anti-porcine FVIII:C Ab.) Hemostatic FVIII:C levels were attained and maintained for 7 d.Conclusions:porcine FVIII is a rational and effective therapeutic alternative for patients with anti FVIII:C Ab; the side effects are acceptable; anamnesis is perhaps less frequent than after human FVIII;however,Ab cross-r ..tivity increases after treatment.

161 CLINICAL EXPERIENCE WITH POLYELECTROLYTE-FRACTIONATED PORCINE F VIII IN THE TREATMENT OF 4 HEMOPHILIACS A WITH INHIBITOR.

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Aim : We evaluated clinical tolerance, in vivo F $VIII_C$ recovery and immunogenic potential of polyelectrolyte-fractionated porcine F VIII (PE porcine F VIII).
Method : 4 hemophiliacs A with inhibitor were treated with PE porcine F VIII (Hyate C) for orthopedic surgery and bleeding. 1 patient has been on prophylaxis (40 U/Kg each 48 hours) ever since February 1982. Controls were : clinical tolerance, clinical efficacy, platelets counts, in vivo F VIII level, anti human F $VIII_C$ and anti porcine F $VIII_C$ inhibitors.
Results : Clinical tolerance was bad in 1 patient, poor in 2 patients, good in 1 patient ; clinical efficacy was good in 3 patients and bad in 1 patient. In vivo F $VIII_C$ recovery was excellent in 3 low responders and bad in 1 high responder. Clinical efficacy was always corelated with the F VIII recovery. We did not observe thrombopenia. In 3 low responders, anamnestic responses were low ; in 1 high responder it was high.
Conclusion : PE porcine F $VIII_C$ in vivo recovery was excellent and clinical efficacy good but the tolerance was bad. In 4 patients immunogenic potential was the same as that we observed with previous human F VIII infusions. We conclude PE porcine F VIII concentrates are indicated for high responders with low crossed immunity for porcine F VIII.

III 6/11/

207 HEPATITIS B VACCINATION IN HEMOPHILIACS. A joint study between the Hemophilia Centers in Leuven, Belgium and Malmo, Sweden

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Aim

To study the immunogenicity of the HB vaccine (MSD) in hemophiliacs and non-hemophiliacs.

Methods

Sixteen Swedish hemoph. (0.5-12 yrs), 5 v Willebrand pat. (2-8 yrs) and 1 girl (3 yrs) with a f XIII def. all lacking HBV markers as well as 8 Belgian hemoph. (4-34 yrs) received 20 ug HB vaccine (MSD) s.c. Twenty Swedish non-hemoph. (8 males, 14 fem.; 2-47 yrs) also were given the vaccine s.c. In Leuven 27 (14 males; 13 fem.) non-hemoph. (22-47 yrs) got the vaccine i.m. and another 26 s.c. (14 males; 12 fem.; 22-44 yrs). All individuals were vacc. on d 9, 30 and 180 and checked as for HB markers and transaminases at the same occasions and also on d 90 and 270. The controls in Leuven also voluntaried additional samples on d 310 and 360. HBsAg, anti-Hbc were determined in Malmö or Leuven and anti-Hbs in Leuven (Ausab-Hollinger method).

Results

No adverse reactions were seen except for a few sore and swollen arms after s.c. vacc. After 2 mths all 7 Belgian hemoph. and 12/13 Swedish ones had anti-Hbs. Three months after the 3rd inj all the Belgian hemoph. had subst. titers of anti-Hbs. The controls showed a somewhat slower response. At 9 months all Belgian non-hemoph. showed a subst. anti-Hbs titer and 13/17 of the Swedish group had an anti-Hbs 3 months after the 1st injection.

Conclusion

HB vaccine was as immunogenic in hemoph. as in normals. Side-effects were minimal but marginally more pronounced by the s.c. than by the i.m. route.

208 RESULTS OF HEPATITIS B SURFACE ANTIGEN VACCINE ASSOCIATED TO SEROPROPHYLAXIS IN HEMOPHILIACS AND PATIENTS WITH OTHER CONSTITUTIONAL COAGULATION DEFECTS.

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Aim: We studied immunogenic effect and preventive efficacy of hepatitis B vaccine (Hevac B Institut Pasteur, Paris) in hemophiliacs and patients with congenital bleeding diseases.

Method : 34 patients have been included in this study : 25 hemophiliacs A and B, 2 hemophilia carriers, 7 patients with VWD. Initial blood specimen were negative for HBs Ag anti HBc and anti HBs (RIA) and $\leq$1500 nkat/l for serum aminotranferase activity (ATA). Patients received 3 subcutaneous injections of vaccine at monthly intervals, then another one after one year. 225 IU of anti HBs plasma (CNTS Paris) was administered intravenously with the first injection of vaccine.

Follow-up : monthly controls over 4 months, then every 6 months for : HBs Ag, anti HBc, titration of anti HBs and ATA.

Results : At the present time, we have collected results in 22/34 patients. All our patients were infused with AHF concentrates during the study. We did not observe HBV infection in 16 hemophiliacs on a yearly period. No side effect was observed. The lowest value of anti HBs, in 22/22 tested patients 4 months after the first injection was 125 mUI/ml. In 22/24 patients, variations of ATA were the same during vaccine period than previously. In 2/24 patients, ATA increased.

Conclusion: Hevac B IPP proved safe immunogenic and effective in patients with congenital bleeding diseases.

265 CLINICAL EVALUATION OF A HEPATITIS-SAFE FACTOR IX CONCENTRATE: M. Einarsson*, E. Hedner**, A. Ljunggvist*, P.M. Mannucci*** and L.O. Nilsson*

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Aim

The present study was designed to clinically evaluate a factor IX concentrate (Preconativ®, KabiVitrum AB, Stockholm) made hepatitis-safe by treatment with octanohydrazide-Sepharose 4B.

Method

Factor IX concentrate was prepared by the DEAE-Sephadex procedure followed by chromatography on octanohydrazide-Sepharose 4B to remove possible hepatitis virus(es).

Results

The concentrate was given in doses of 20-40 U IX:C/kg b w. Three patients (2 with severe haemophili B) without any sign of previous hepatitis infection were treated, one of them at 3 separate occasions. There was no evidence for hepatitis infection in any of the patients during the 6-9 months of follow up. Six other patients (5 severe and 1 mild haemophili B) were also treated. The patient with mild and one with severe haemophili were treated at 2 different occasions. No clinical hepatitis has developed so far (3-6 months after the given treatment). In none of the patients did any increase of the S-ALAT level occur. The in vivo recovery varied between 23 and 58% (mean 47%). The hemostatic effect was good and no immediate side effects were seen. The half-life of IX:C was calculated (3 pat.) and found to be similar to that of Preconativ (T/2 22 h).

Conclusions

Preconativ treated by octanohydrazide-Sepharose 4B has been given to 9 patients so far without any subsequent rise in S-ALAT. The in vivo recovery and half-life was similar to that found for ordinary Preconativ. The hemostatic effect was good and no immediate side-effects were seen.

0319

PURIFICATION AND CHARACTERISATION OF HUMAN FACTOR VIII:C

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The Haemophilia Centre, Academic Department of Haematology, Royal Free Hospital, London, England (1), and Speywood Laboratories, Nottingham, England (2).

Factor VIII:C, the coagulant principle lacking from the plasma of the commonest type of haemophilia has been very resistant to attempts at purification. A new scheme, incorporating monoclonal antibodies was devised as follows:- 1 kg batches of human plasma cryoprecipitate were adsorbed to polyelectrolyte ES then eluted and passed over a column containing monoclonal antibody to von Willebrand factor covalently linked to sepharose. This column adsorbed the whole factor VIII complex and after washing was eluted with .24 M $CaCl_2$, detaching VIII:C but leaving VIII:Ag in the stationary phase. The eluate was directly applied to a column of sepharose linked monoclonal antibody to factor VIII:C, to which it bound tightly. After elution the protein had a specific activity of 4,000 to 5,000 u/mg. Despite carrying out the procedure continuously over 24 hours in the presence of 1-2 mM DFP there was evidence of variable proteolysis in the end product. The predominant band observed on SDS polyacrylamide gel electrophoresis developed with silver stain was a single chain species Mr 365, K. In some purifications using older batches of cryoprecipitate other bands appeared at around 275 K and 80 K. The higher molecular weight bands were positive on immunoblotting with a different monoclonal to that used in the purification. A unique amino acid sequence has been determined for the terminal of the 80 K fragment.

111 6718

0320

THE EFFECTS OF THE COOLING RATE DURING FREEZING OF PLASMA ON SOLUTE CONCENTRATION AND FACTOR VIII RECOVERY IN CRYOPRECIPITATE. J.Over, J.Oh, H.Henrichs, C.C.Ouivis-Vorst, and J.A.Loos, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

Freezing of blood plasma is an essential step in the production of Factor VIII preparations. However, little is known of the physical changes during freezing and their effect on Factor VIII. We studied the factors influencing the cooling rate of plasma in blood bags, the changes in solute concentrations at different cooling rates and the effect of salt on cryoprecipitation of Factor VIII. Four cooling regimes with freezing times of a few minutes to several hours were applied to bags filled with varying volumes of saline and plasma. The freezing time was inversely proportional to the surface area and directly proportional to the filling of the bag. Four different sampling techniques showed that during slow freezing of saline NaCl accumulated in the centre of the bag (conc. up to 1,5 M). When frozen within 30 minutes no accumulation was found. When freezing plasma, albumin, IgM and total protein showed a pattern not significantly different from that of NaCl. Factor VIII did not accumulate in the centre during slow freezing, partly due to spontaneous cryoprecipitate formation all over the bag.

Another factor influencing Factor VIII recovery in cryoprecipitate was the salt concentration. Cryoprecipitation of plasma to which varying amounts of NaCl had been added showed a decrease in Factor VIII recovery with increasing salt concentrations, whereas protein precipitation was promoted, resulting in low specific activities of Factor VIII.

Finally, it was shown that rapid freezing of normal plasma to which no salt had been added resulted in a higher recovery and specific activity compared to slow freezing.

It is concluded that during freezing of plasma salt and protein gradients may form dependent on the rate of cooling. When gradients are formed, they influence the Factor VIII recovery in cryoprecipitate by causing dissociation of Factor VIII coagulant activity from the complex. Factor VIII cryoprecipitation appears to be primarily a cold-induced process not based on salting-out effects.

0321

PURIFICATION OF HUMAN FACTOR VIII PROCOAGULANT PROTEIN (FVIII). Rob J. Hamer, Nel H. Beeser-Visser, Jan J. Sixma, Dept. of Haematology, University Hospital Utrecht, The Netherlands.

A simple, three step procedure was developed for human Factor VIII which yielded a homogeneous preparation with coagulant activity.

1. Factor VIII-von Willebrand Factor (FVIII-VWF) from Hyland Intermediate Purity Concentrate was chromatographed on a Sepharose 6B column in the presence of Dextran. A homogeneous FVIII-VWF preparation was obtained with a specific coagulant activity of 170 U/mg at a yield of 35-40%. FVIII was separated from VWF using Dextran Sulphate Sepharose.
2. FVIII was separated from VWF using Dextran Sulphate Sepharose. FVIII was eluted stepwise at 255 mM $CaCl_2$, pH 7.0, while VWF remained bound. This yielded a preparation with a specific coagulant activity of > 1200 U/mg at a recovery of 50%.
3. Remaining contaminants were removed with Fast Protein Liquid Chromatography using a Mono-Q column. FVIII was eluted with a 50-250 mM $CaCl_2$ gradient, pH 6.0. This step has up till now only performed in small scale experiments. A FVIII preparation was obtained containing 2-4 U at a recovery of 60% without detectable protein. A single broad band with a M_r of 82-85.000 was found with SDS-polyacrylamide gradient gel electrophoresis (3-30%) of a reduced preparation followed by ultrasensitive silver staining. This band disappeared in favour of a band with a M_r of 70.000 after thrombin treatment.

0324

HIGHLY PURIFIED AND HEAT-STERILIZED CONCENTRATES OF FACTOR IX AND PPSB. N.Heimburger, H.E.Karges and G.Kumpe, Research Laboratories of Behringwerke, Marburg, West Germany.

Applying modern separation techniques for the fractionation of plasma, concentrates of the most important clotting factors can be prepared. The broader application of these concentrates, however, revealed two serious side effects: the transmission of serum hepatitis and, especially in the case of the prothrombin complex, the thrombogenicity. Since several years worldwide negotiations are undertaken to eliminate those risks for the patients. By improved fractionation techniques we have succeeded to eliminate contaminating proteins as well as hepatitis markers; in addition we were able to sterilize the products by heat analogous to albumin. Adding deliberately HBsAg to the source material for fractionation we were able to show that the fractionation method used is able to eliminate around 10^6 HBs-antigen particles. To increase the hepatitis-safety further, we have included a sterilization step - heating to 60°C for 10 hours in solution in presence of stabilizers - in the preparation procedure. The efficacy of this step has been shown three times in the chimpanzee model using other proteins like F VIII or F XIII. This heating procedure probably also eliminates the risk of transmission of non-A-non-B-hepatitis, however, this can only indirectly be proved because test methods are lacking. The purity of the products is characterized by the specific activity; it amounts to 9 IU/mg in the case of F IX-concentrate and 3,5 IU/mg in the case of PPSB-concentrate. Conventional concentrates, for comparison, have specific activities around 0,5-1,5 IU/mg protein. The nativity of the new products, measured by the ratio of activity to antigen, is also better than with conventional concentrates: for F IX in PPSB concentrates the ratio is 0,525 in comparison to conventional concentrates with a ratio of 0,33. As a reason for the thrombogenicity activated clotting factors are accused. In conventional PPSB-concentrates of different manufactures thus an average of 11 mU/ml Kallikrein can be found; in contrast, our new PPSB-concentrate shows only about 1,1 mU/ml. The efficacy of the heating step to inactivate infectious HBV is tested in monkeys.

0353

A SINGLE STEP METHOD FOR THE ISOLATION OF ANTITHROMBIN III. M. Wickerhauser and C. Williams, American Red Cross Blood Services, Plasma Derivatives Laboratory, Bethesda, MD, 20814, USA.

We previously described a 3-step method for large scale isolation of antithrombin III (AT III) from cryosupernatant that includes affinity chromatography on heparin-Sepharose (HS), precipitation with polyethylene glycol (PEG) 4000 and rechromatography on HS to eliminate the PEG reagent (Wickerhauser and Williams, Thromb. Haemost. 42:168, 1979). The PEG step served to precipitate some impurities including HBsAg if present in the starting material. However, after the report of a successful inactivation of HB viral infectivity during pasteurization of AT III concentrate (Tabor et al. Thromb. Res. 22:233, 1981) the need for the PEG step to reduce the risk of hepatitis has diminished. Our studies have shown that the PEG step and consequently the second HS affinity chromatography step can be replaced without any significant effect on purity or yield of the final product, by increasing the salt concentration of the HS-washing buffer from 0.5 to 0.6 M NaCl to enhance removal of impurities prior to elution of the AT III protein with 2 M NaCl. In an initial experiment, starting from 1000 ml of plasma cryosupernatant, we obtained AT III at a yield of 32% with a specific activity of 7.3 plasma equivalents per mg protein. On polyacrylamide gel electrophoresis the AT III was judged to be over 95% pure. This single step method should be more economical for the large scale preparation of AT III for clinical use and the shorter time required for production should significantly reduce the risk of pyrogen development.

1024

ANTITHROMBIN III CONCENTRATION AND ANTITHROMBIN III SUBSTITUTION IN EXPERIMENTAL DISSEMINATED INTRAVASCULAR COAGULATION. G. Müller-Berghaus (1), N. Niepoth (1), B. Rabens-Alles (1), E. Rump (1), and G. Murano (2). Clinical Research Unit for Blood Coagulation and Thrombosis, Max-Planck-Gesellschaft, Giessen, West Germany (1), and Division of Blood and Blood Products, FDA, Bethesda, MD, USA (2).

Since AT III is thought to be the main physiological inhibitor of coagulation in plasma, the importance of this protein was investigated in a controlled model of disseminated intravascular coagulation (DIC). Rabbits were injected with 2 doses of endotoxin (S. enderitidis: 50 µg/kg) and treated either with AT III concentrates (25 u/kg/h), heparin (50 u/kg/h), AT III and heparin, or saline. 19 animals injected with endotoxin and treated with saline demonstrated intravascular coagulation (drop in platelet counts and fibrinogen concentrations) and renal glomerular microclots. In these animals, however, neither AT III activities nor AT III concentrations decreased. If DIC was prevented by high doses of heparin (200 u/kg/h) AT III activities and concentrations did not change. Small doses of heparin (50 u/kg/h) prevented DIC in 50 % of the animals. If rabbits were treated with small doses of heparin (50 u/kg/h) and additionally infused with human AT III concentrates, DIC could be completely prevented. These experiments demonstrate that human antithrombin III potentiates the inhibitory effect of heparin in vivo. The failure to show a decrease in AT III concentrations indicates that only very small, but not measurable amounts of AT III are necessary for mediating the inhibitory effect of heparin in rabbits, or that AT III is not involved in limiting experimental DIC. The drastic drop of AT III observed in patients with DIC might be caused by other mechanisms.

1081

CANINE MODEL OF HEMOPHILIA WITH ANTIBODIES TO FACTOR VIII:C. A.P. Giles, S. Tinlin, P. Greenwood, H. Hoogendoorn and R. Greenwood. Departments of Pathology, Medicine and Animal Care, Queen's University at Kingston, Ontario, Canada.

Congenital Factor VIII deficiency was diagnosed in a pure bred miniature Schnauzer (MS) dog. Further investigation and breeding studies confirmed that the condition was a precise counterpart of human hemophilia in terms of inheritance pattern and clinical and laboratory abnormalities. Using a pure bred MS affected male, a breeding program was initiated and a colony established. First generation animals were obtained either from cross-breeding with a normal Brittany spaniel (BS) female or inbreeding with a carrier pure bred MS female. Obligate cross-bred carriers from the former and a hemophilic female MS from the latter were further bred to a single normal male beagle to produce a third generation of hemophilic males and females. All the hemophilic animals were treated with canine cryoprecipitate for joint bleeds, hematomas, etc. Four animals developed potent anti-Factor VIII:C antibodies. All belonged to the breeding arm resulting from the mating of the normal BS female to hemophilic MS male. None of the animals in the other arms of the breeding program have developed antibodies. The mean dose of Factor VIII transfused (Tx) in the inhibitor animals was 286.25 ± 156.12 (SD) and the age at 1st Tx 7.30 ± 7.21 wks. The antibodies have been characterized as IgG, Type II in their mode of inactivation and recognize both human and canine but not porcine Factor VIII:C in vitro. A massive intra-abdominal bleed in one animal was treated successfully with porcine Factor VIII concentrate (Hyate) and controlled studies using a standardized measure of bleeding confirmed this product's efficacy. All animals receiving porcine Factor VIII subsequently developed antibodies to porcine VIII:C but these appear saturatable in contrast to those to canine VIII:C. This animal model of hemophilia with the propensity to develop antibodies in 1 generation offers a unique opportunity to study both the pathogenesis and management of this complication of hemophilia. The breeding studies confirm the apparent inherited predisposition for this condition and the relationship of antibody development to a breed other than that in which hemophilia was diagnosed is of great interest.

1096

PREVALENCE OF ISOLATED PROTEIN C DEFICIENCY IN PATIENTS WITH VENOUS THROMBOTIC DISEASE AND IN THE POPULATION. A.W. Broekmans, L.E. van der Linden, J.J. Veltkamp, R.M. Bertina. Hemostasis and Thrombosis Research Unit, Department of Internal Medicine, Leiden University Hospital, Leiden, The Netherlands.

Isolated protein C deficiency is associated with venous thrombotic disease. In order to estimate the prevalence of isolated protein C deficiency we studied two groups of patients selected out of all patients (n = 800) treated for venous thrombo-embolism with vitamin K antagonists by the Leiden Thrombosis Centre at May 1, 1982. In the first group (A: n = 62) all patients younger than 41 years with either superficial thrombophlebitis, deep venous thrombosis, and/or pulmonary embolism were included. The second group (B: n = 257) contained all patients older than 40 years with recurrence(s) of venous thrombo-embolism.

The laboratory diagnosis of protein C deficiency was made on basis of three criteria previously described (Thromb Haemost 1982;48:1-5). All patients with isolated protein C deficiency were studied at 2 different occasions. In group A, 5 patients (8.1%) fulfilled the criteria for an isolated protein C deficiency, one of these patients was not stably anticoagulated. In group B, 3 patients (1.2%) fulfilled the criteria for an isolated protein C deficiency.

These data indicate that the frequency of isolated protein C deficiency in patients with venous thrombo-embolism is at least 1%. Considering the population that is covered by the Leiden Thrombosis Centre (400,000) and the previous observation that only 1 out of 3 patients with an isolated protein C deficiency is on oral anticoagulant treatment, it is possible to estimate that the prevalence of isolated protein C deficiency in the Western part of The Netherlands is about 1 per 16,000.

1126

HUMAN ANTITHROMBIN III (AT III) PROTECTS RABBITS FROM ENDOTOXIN INDUCED INTRAVASCULAR COAGULATION. D.C. Triantaphyllopoulos. American Red Cross Blood Services, Plasma Derivatives Laboratory, Bethesda, MD, 20814, USA.

In previous work we showed that preinjection of AT III into normal rabbits infused with tissue factor does not significantly affect mortality or the decline in coagulation parameters (Fed. Proc. 40:1028, 1981). In the following experiments we investigated the effectiveness of AT III in intravascular coagulation induced by the infusion of endotoxin. Twenty-one rabbits were infused with 20 µg/kg/hr of E. coli endotoxin through the marginal vein of the ear for 6 hrs. Eight of the animals were preinjected through the corresponding vein of the opposite ear, immediately before the infusion of endotoxin, with a bolus dose of human AT III calculated to increase the antithrombin content of the plasma by about 3 units/ml. Blood samples were obtained from the carotid artery through a polyethylene catheter: (A) before the infusion of endotoxin or the injection of AT III and (B) 2, 4 and 6 hrs after the start of the infusion of endotoxin. All eight animals which were preinjected with AT III survived, while 5 of the 13 control rabbits (not preinjected with AT III) died. This difference in mortality was statistically significant ($P: < 0.05$). The changes in coagulation parameters from the baseline values were compared between the 8 control rabbits which survived and the 8 animals which were preinjected with AT III. The concentration of the preinjected human AT III declined significantly faster ($P: < 0.01$) than that of the native rabbit antithrombin. AT III prevented the decline in F.XII throughout the infusion of the endotoxin and the decline of F.VIII during the first 2 hrs of the infusion ($P: < 0.05$). However, the decline in F.V, fibrinogen, prothrombin and platelets was not affected ($P: > 0.5$) by the injection of AT III. In conclusion AT III prevents the death which is induced by E. coli endotoxin in rabbits by partially counteracting the coagulation abnormalities.

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0362

TURNOVER STUDIES OF RADIOLABELLED ANTITHROMBIN III IN LIVER DISEASE.

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Metabolism of purified human Antithrombin III (specific activity 5.3 U/mg, endotoxin free, single band on SDS gelelectrophorese, highly purified according to amino-acid sequence analysis) was studied in 9 patients with severe liver disease and 6 normal subjects. The antithrombin III was labelled with ^{125} Iodine (McFarlane technique) without any change in biological activity and injected intra venously. All patients had stable liver cirrhosis without signs of overt ascites. In all patients liver biopsy showed a histopathological micronodulair type. Mean antithrombin III levels in plasma were 46,2 U/ml in patients and 98.5 U/ml in normals. All patients had normal fibrinogen and FDP levels and none of them had a positive E.G.T. Albumin concentrations in patients were 30.3 gr/L (n=38 gr/L).
The B half life varied between 42,8and 72,4 hours (mean 57.5 hours) in the patients and between 54,0 and 78,0 hours (mean 63,1 hours) in normal subjects. ($p > 0.05$)
K 1,0 (fractional catabolic rate) of patients and normal subjects showed no difference. Two patients with a short half life of 44,7 and 42,8 hours had larger central compartment.
These results suggest that the lowered AT-III plasma levels in cirrhotics are mainly due to decreased synthesis.

0363

DYSFUNCTIONAL ANTITHROMBIN III IN SICK PREMATURE INFANTS.
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Sick newborn infants are at risk of developing thrombotic complications. Antithrombin III (AT-III), a major inhibitor of coagulation has low immunologic levels in the newborn which vary significantly with the gestational age (GA), postnatal age and health status of the infant (Andrew et al, Ped. Res. 16,4(II):275A, 1982). The objective of this study was to determine if the AT-III molecule is fully functional in sick premature infants. The populations studied included: adult controls (n=20), fullterm healthy infants (n=18), sick premature infants (≤37 wks GA) on day 1 (n=14), and ≥14 days (n=10), and infants with disseminated intravascular coagulation (DIC) (n=6). Sick infants had a variety of disorders including respiratory distress syndrome (RDS) (79%), sepsis (45%), birth asphyxia (21%) and congenital heart disease (14%). DIC was diagnosed in the presence of a prolonged prothrombin time, partial thromboplastin time, thrombin time; decreased fibrinogen level, and platelet count; and elevated fibrin degradation products. Plasma AT-III levels were measured biologically (B) (chromogenic substrate S2238) and immunologically (I) (radialimmunodiffusion). Levels are expressed as a percent of adult pooled-plasma (mean ± SEM).

AT-III	Adults	Fullterm	Sick Premature	
			Day 1	Day 14
Biologic	100 ± 2	54 ± 4	28 ± 4	41 ± 7
Immunologic	102 ± 2	57 ± 2	35 ± 3	56 ± 5
B/I ratio	.97 ±.02	.95 ±.1	.85 ±.1	.72 ±.1

Sick premature infants without DIC had significantly less biologic activity compared to immunologic levels by 1- days of life ($P<0.01$). All of these infants required ventilatory support for respiratory problems (RDS, bronchopulmonary dysplasia). DIC depressed I and B levels of AT-III in both preterm (B=17±3.2, I=21±3.5, B/I=0.78±11, n=9) and fullterm infants (B=19±7.8, I=35±5.1, B/I=1.1±0.1n, n=1). We conclude that some chronically ill premature infants have dysfunctional AT-III.

0302

FACTOR VIII BINDING TO PURIFIED PHOSPHOLIPIDS.
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Previous work has shown, using the Factor VIII clotting antigen (F VIII C:Ag) assay and sucrose density gradient ultracentrifugation (SDGU), that Factor VIII clotting protein binds with high affinity to phospholipid (PL) vesicles and that this binding may protect F VIII clotting activity (F VIII:C) from inactivation by human antibodies. This interaction has been studied further using the F VIII C:Ag assay, thrombin generation of F VIII/PL mixtures in inhibitor haemophilic plasma (IHP) and SDGU of F VIII/PL.

Binding of F VIII C:Ag to PL vesicles is shown by a reduction in the measured F VIII C:Ag in a sample of F VIII, after incubation with PL. Of several purified PLs used, only phosphatidyl-serine (PS) and phosphatidic acid showed significant binding to F VIII C:Ag. Phosphatidyl-choline (PC), -ethanolamine and -inositol were ineffective. In addition, when PC/PS vesicles of varying compositions were used, F VIII C:Ag binding to PL varied with PS content and PL concentration: below 15% PS, F VIII C:Ag binding was negligible at PL concentrations. At high PL concentrations (250 ug/iu VIII) C:Ag binding was optimal at all PS levels over 20%. At lower PL concentrations, C:Ag binding increased as the PS level rose from 15 to 100%.

When F VIII was incubated with similar mixtures of purified PLs, then added to IHP and recalcified, only mixtures containing PS were able to generate significant quantities of thrombin.

SDGU of purified F VIII:PL mixtures, using PC/PS (2:1) vesicles as PL, showed that F VIII:C was recovered in the upper portion of the gradient together with the PL vesicles, while F VIII-related antigen (F VIII R:Ag) was present only in denser fractions. In the absence of PL, F VIII:C and F VIII R:Ag sedimented together in the dense fractions.

These studies suggest that a specific interaction occurs between Factor VIII clotting protein and certain purified PLs, especially PS. This interaction causes dissociation of F VIII:C from F VIII R:Ag and is able to 'protect' F VIII:C from rapid inactivation by human anti-F VIII:C.

0334

MANAGEMENT OF IMMUNOLOGIC THROMBOCYTOPENIC PURPURA IN PREGNANCY BY HIGH-DOSE INTRAVENOUS IMMUNOGLOBULIN G.
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The pathogenesis of immunologic thrombocytopenic purpura (ITP) is explained by antiplatelet autoantibodies of which the transplacental passage was demonstrated. In pregancy both mother and fetus are endangered by bleeding complications. We report on 4 cases of ITP found in week 20 - 39 of gestation; the platelet counts ranged from 4000 to 16000/µl. In all patients there was little or no response to prednisone (1 mg/kg b.w.). As introduced in pediatric therapy by IMBACH et al. the mothers were placed on high-dose intravenous immunoglobulin G (0.4 g/kg b.w.) on five consecutive days. Two of the patients showed a rapid increase of the platelets beyond 100000/µl. The two others achieved a platelet count (67000 and 34000 resp.) sufficient for vaginal delivery and cesarean section. When vaginal delivery was performed, fetal platelets were measured during delivery. Transplacental immunoglobulin treatment of the fetus was evidenced in one newborn by the platelet profile postpartum. The resulting thrombocytopenia was treated successfully by gammaglobulin infusions (0,4 mg/kg b.w.). In one mother thrombocytopenia relapsed three weeks after the immunoglobulin therapy. The platelet counts of two others remained stable after the delivery and one patient responded to prednisone. Complications in mothers or infants from immunoglobulin treatment were not observed.

In contrast to the conventional therapy with corticosteroids and/or splenectomy high-dose intravenous immunoglobulin application seems to offer a new and safe way to increase maternal and fetal platelet counts without side effects.

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