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now free from hepatitis risk:
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Until recently it has been impossible to eliminate the danger of hepatitis from certain plasma products, in particular clotting factor concentrates. When using Factor VIII concentrate for haemophilia it was therefore necessary to weigh the benefits against the hazards. Now however, thanks to a new manufacturing process, a safe Factor VIII concentrate is available. Experimental and clinical trials have confirmed its freedom from hepatitis risk.

Haemophiliacs, because they require life-long replacement therapy with coagulation factor concentrates, are exposed to considerable risks of hepatitis. Twenty years ago, before the introduction of effective replacement therapy, haemorrhage was the major hazard, but today its place has been taken by chronic liver disease.

Several studies have demonstrated that the incidence of icteric hepatitis among patients with haemophilia is of the order of 15%¹ (Tab. 1). Assuming that anicteric cases are of equal frequency, the total incidence of hepatitis must be of the same order as that seen after transfusions of 6 or more units of blood^{2,3}. The true figures are probably considerably higher, but the only way of ascertaining them was to investigate haemophiliacs who were receiving replacement therapy with Factor VIII for the first time. In such patients hepatitis rates of 60% have been found¹.

Radioimmunological techniques for detecting the markers of hepatitis B virus have shown that all patients receiving replace-

ment therapy have in fact been exposed to the virus (Tab. 2).

In the Heidelberg Haemophilia Centre 7% the patients were found to be HBsAg-positive and 88% had antibodies against HBsA that gives a total of 95%¹. A report from Munich, covering 55 children with haemophilia showed that anti-HBc is probably a still more sensitive marker for hepatitis B infection, as revealed a prevalence of 96.1%.

As yet there have been no systematic investigations of the prevalence of non-A/non

Investigators	Number of patients	Number with hepatitis
Mannucci	74	14
Schimpf	88	13
Tilsner	62	2

Tab. 1 Cases of icteric hepatitis (i. h.) among haemophiliacs

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Investigators	Anti-HBc	Anti-HBs	Total
Hasiba 1977	69%	80.5%	88.5%
Schimpf 1976	7%	88%	95%
Hilgartner 1977		90%	92%
Cederbaum 1978	4%	70%	75%

Tab. 2 Hepatitis B markers (RIA) in haemophiliacs

hepatitis among haemophiliacs, but from the increasing number of case reports it is apparent that here, too, there has been a shift in the virus spectrum similar to that which has occurred in posttransfusion hepatitis, Type B having been partly replaced by Type non-A/non-B. The latter form has proved especially dangerous among patients with haemophilia, as it may occur despite the existence of immunity to hepatitis B⁵ and frequently runs a chronic course. Repeated attacks in the same patient have been reported^{6,7}. At present there are no specific markers for detecting non-A/non-B hepatitis. The diagnosis is made by excluding other known causes of hepatitis.

The risk of hepatitis among haemophiliacs had become very serious and urgent measures were required to combat it. In principle there were two possibilities:

1. Active or passive immunization of haemophiliacs.

2. Introduction of products free from hepatitis risk.

Vaccines and immunoglobulins against hepatitis are at present available only for hepatitis B and only in limited amounts.

Suggestions that haemophiliacs should be treated with single donor cryoprecipitate derived from medically supervised regular donors would have solved the problem only for a very small number of patients.

Previous attempts to reduce the infectivity of clotting factor concentrates had been confined to Factor IX. By a process called «cold sterilization» – a combination of β -propiolactone treatment and ultraviolet irradiation – it had proved possible to produce PPSB preparations of diminished infectivity⁸. This process was, however, not applicable to Factor VIII.

In view of these facts we endeavoured to work out a method for producing hepatitis-free Factor VIII concentrate. We chose heat sterilization, because it had been used for albumin for many years and was of established value. The removal of hepatitis risk by the albumin production process is based essentially on three stages: 1. Screening of all donor plasmas by a third generation test and rejection of HBsAg-positive donations. 2. Elimination of hepatitis virus (HV) during the fractionation process. 3. Inactivation of any residual virus particles by heating the final

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Production steps	Decrease in experimentally added HBV		Specific activity (U/mg protein)	Chimpanzee infectious dose
	(ng/ml)	(CID ₅₀)		
1 Pooled cryoprecipitate	90	10 ^{4.5}	0.3	
Ad-sorption				
2 Supernatant	75	10 ^{4.5}	0.3	
Precipitation				
3 Supernatant	75	10 ^{4.5}	1.5	
Precipitation and solution				
4 Factor VIII concentrate	≤ 1	≤ 10 ^{2.5}	5	10 ^{4.5}
heat to 60°C for 10 hours in the presence of stabilizers	< 1	< 10 ^{2.5}	10	
5 Heated Factor VIII solution	< 1	< 10 ^{2.5}	20	
Precipitation, solution and dialysis				
6 Factor VIII HS	< 1	< 10 ^{2.5}	6	

* Tested by RIA (HBsAg) ** Estimated from HBsAg assay *** Lower limit of detection by RIA = 1 ng HBsAg/ml **** Chimpanzee Infectious dose

Fig. 1 Experiments showing the decrease in concentration and inactivation of HBV during the production of Factor VIII HS Behringwerke

product to 60°C for 10 hours. The Factor V molecule is highly susceptible to elevated temperatures and the heating process was made feasible only by addition of stabilizers which protect the molecule from thermal inactivation.

The first tests to demonstrate the safety of the process used for producing Factor VIII HS* were carried out in chimpanzees. In the first instance they were limited to hepatitis virus, because standardized infective material and specific markers for detecting the disease are available for this virus type only.

By adding hepatitis B virus (HBV) with a titre of known infectivity to the original cryoprecipitate it proved possible to demonstrate that the concentration of hepatitis viruses was reduced by a factor of 10² during the first steps of the production process and before the heating stage: expressed in CID₅₀/ml fell from 10^{4.5} to 10^{2.5} (Fig. 1).

Proof that the heating step is essential for producing a hepatitis-free preparation was obtained by experiments in chimpanzees. In four chimpanzees which were given 1 ml non-heated Factor VIII concentrate with experimental HBV content of ≤ 1 ng/ml equivalent to a CID₅₀/ml of ≤ 10^{2.5} (lower limit of detection by RIA), had attacks of hepatitis B (Tab. 3, Fig. 2).

* Factor VIII HS (hepatitis-safe), Behringwerke AG, Marburg

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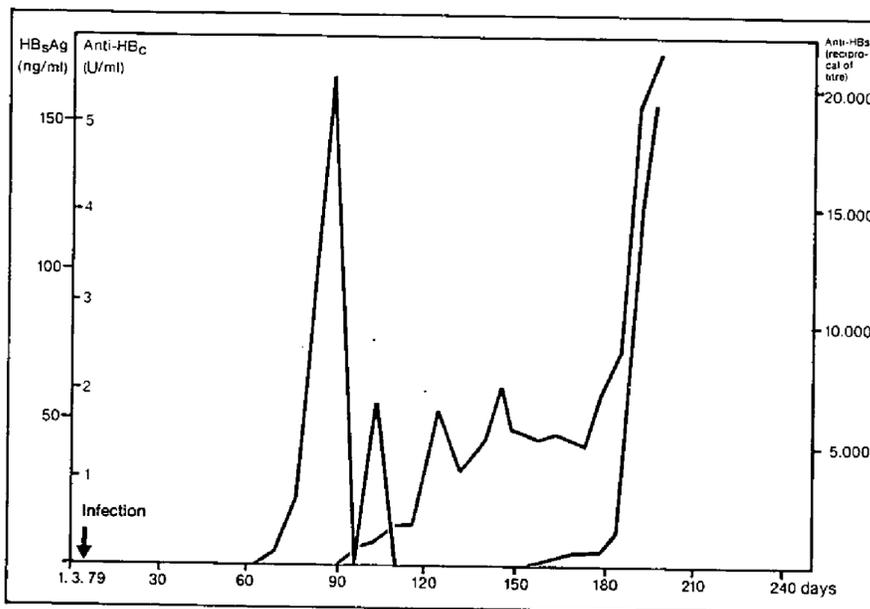


Fig. 2 Typical hepatitis B parameters in an infected animal (chimpanzee 9)

The concentrate which had been heated in solution to 60°C for 10 hours was no longer infectious: the four chimpanzees which were given the heated material intravenously showed no clinical signs of hepatitis B; furthermore, there was no rise in the titres of HBsAg, anti-HBs or anti-HBc; bilirubin, SGPT and SGOT remained within the normal range, and liver biopsies carried out at 2-week intervals showed no abnormalities. As the chimpanzees also remained free from non-A/non-B hepatitis, and as the concen-

trate used for the experiments had been manufactured from pooled plasma, it seems reasonable to assume that any non-A/non-B hepatitis viruses had likewise been eliminated and inactivated.

The material used for the production of Factor VIII concentrate consists exclusively of HBsAg-negative plasma, i.e., the initial plasma contains ≤ 1 ng HBsAg/ml. Processing reduces this by a factor of 100, and the additional heating stage (Fig. 1) inactiva-

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Factor VIII		Sex	Body weight (kg)	Anti-HBc max titre (i.u/ml)	Anti-HBc max titre Index	Anti-HBc max titre Index	Transaminases
Not heated	Chimp. No 1	♂	60	80	21 (+)	55 (+)	+
	Chimp. No 4	♀	50	80	26 (+)	30 (+)	+
	Chimp. No 9	♂	60	80	10 (+)	55 (+)	+
	Chimp. No 17	♂	60	80	16 (+)	35 (+)	+
Heated	Chimp. No 3	♂	50	-	11 (-)	14 (-)	-
	Chimp. No 8	♀	35	-	12 (-)	11 (-)	-
	Chimp. No 10	♂	45	-	10 (-)	13 (-)	-
	Chimp. No 18	♂	50	-	12 (-)	11 (-)	-

* Positive at an index >2.1

Tab. 3 A long-term trial in chimpanzees to demonstrate freedom from active hepatitis B virus after administration of non-heated or heated Factor VIII concentrate (see Fig. 1). Results of serological monitoring over a 9-month period

tes any viruses which may still be present, the final outcome being a product free from hepatitis B risk. Proof that this applies also to non-A/non-B hepatitis must await further clinical observations.

Factor VIII concentrate HS was employed for the prevention and treatment of haemorrhages in a 2-year clinical trial in 44 patients, comprising 40 patients with haemophilia (haemophilia A), two female carriers of haemophilia, one patient with circulating inhibitors to Factor VIII and one patient with von Willebrand's syndrome. The aim of the trial was to test the product for its

efficacy, side-effects and freedom from hepatitis risk.*

During 271 courses of treatment a total of 232,000 units of Factor VIII from 20 different manufacturing batches was employed. To investigate the incidence of side-effect and the rise in Factor VIII 25 patients were given a single injection only.

* Date of assessment: June 1980. Since that time further patients have been treated with Factor VIII HS Behringwerk and in particular several major surgical operations have been performed. The observation period for freedom hepatitis now considerably longer.

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Patient No	Treatment			Monitoring for hepatitis safety			
	Number of applications	Time (U)	Period of treatment (d)	Duration of clinical and serological monitoring (months)	Number of tests (GPT)	Number of tests (U/I)	U/I
1	4	200	4	9	3	3	10
2	6	300	5	11	3	3	10
3	8	600	17	6	4	4	10
4	1	250	1	6	1	1	10
5	36	250	351	12	5	5	10
6	14	250	122	9	4	4	10
7	4	250	3	9	3	3	10
8	55	250	241	9	5	3	10
9	1	250	1	6	1	5	10
10	60	250	300	12	6	6	10
11	24	250	101	4	6	6	10
12	5	250	4	6	3	3	10
Total	218			Mean observation period $\bar{x} = 7.8$ months	$\bar{x} = 3.6$	$\bar{x} = 3.9$	

Tab. 4 Treatment with Factor VIII HS and monitoring for hepatitis safety

Long-term monitoring for signs of hepatitis was carried out in 12 patients who were kept under observation for at least 6 months (Tab. 4). This group consisted of patients who were being treated with Factor VIII for the first time. Before treatment, all the parameters for hepatitis B were negative and there was no clinical evidence of hepatitis.

The clinical efficacy of Factor VIII HS was investigated in 15 patients with acute haemorrhages (haemathroses, haematomas, retinal haemorrhage, bleeding into the lower lip, muscle haemorrhages, intestinal haemorrhage, bleeding after dental extraction).

The product was also employed in connection with six surgical operations (dental extraction, removal of adenoids, thyroidectomy, total hip replacement). In all cases the clinical efficacy was good.

In the patient with a circulating F VIII-antibody treatment was unsuccessful because of the high antibody level.

The recovery and rise in Factor VIII were followed in the course of routine clinical investigations in 35 patients after 48 treatments.

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Factor VIII recovery* was calculated from the following formula:

$$\text{Factor VIII recovery} = \frac{\text{Measured rise in Factor VIII} \times 100}{\text{Theoretically expected rise in Factor VIII}}$$

$$\text{Theoretical rise in Factor VIII} = \frac{\text{Amount of Factor VIII injected} \times 100}{\text{Plasma volume}}$$

$$\text{Plasma volume} = \text{kg body weight} \times 41$$

In 25 patients without haemorrhages an average in-vivo recovery of 71.6% (s = 25.6) was calculated from 31 determinations. The mean rise in Factor VIII per Factor VIII unit administered per kg body weight in these patients was 1.75% (s = ± 0.62).

In 10 patients with haemorrhages (17 determinations) the recovery was, as expected, somewhat lower. On average it was 59.5% (s = ± 37.5%) and the mean increase in Factor VIII per unit/kg body weight was 1.45% (s = ± 0.92).

The mean plasma half-life was measured in 4 patients (9 determinations) and amounted to 8.1 hours.

Bearing in mind that these determinations were simply routine assays and were not performed as kinetic measurements by a single phase or two-phase test which would have yielded maximum values, it is clear that these results are of the order of magni-

tude to be expected from Factor VIII concentrates^{9, 10}.

Factor VIII HS was well tolerated in all cases; no allergic reactions were observed. Fluctuations in body temperature and blood pressure remained within the biological range of variation. The platelet count determined in 24 patients before and after Factor VIII administration; it remained unaffected.

Long-term monitoring for hepatitis serology was carried out in 12 patients who met criteria for acceptance mentioned above. They included four infants about one year old and four children from 2 to 10 years. They received in all 218 administrations with a total of 172,000 units of Factor VIII HS. Two young infants with severe haemophilia were given 55 and 60 prophylactic doses respectively, the corresponding total of Factor VIII HS being 40,000 and 50,000 units.

Laboratory monitoring over 6 to 12 months showed no evidence of hepatitis infection. The transaminases SGOT (39 determinations) and SGPT (43 determinations) were all within the normal range. Only in one case, Patient 9, a 40-year old female carrier, was there slight elevation of GPT values. However, hepatitis was excluded as a cause of the rise in transaminases (Table 1).

Tests for HBsAg, anti-HBs and anti-HBe were carried out on 59 occasions and proved negative in all patients. These results provide valuable confirmation of the c

* Recovery = measured rise in Factor VIII expressed as a percentage of the theoretically expected rise.

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	Factor VIII concentrate Behringwerke	Factor VIII HS Behringwerke	Factor VIII concentrate
Factor VIII activity (U/ml)	25	25	25
Protein content (mg/ml)	4.9	4.1	14.6
Specific activity (U/mg protein)	5.1	6.1	1.7
Factor VIII R:Ag/ Factor VIII:C	3.0	3.0	8.5
Factor VIII R:COF (U/ml)	23.6	21.0	11.6
Fibrinogen (mg/ml)			
- functional assay by Clauss method		-	5.6
- immunological assay by radial immunodiffusion	0.6	-	6.0
Fibronectin (ClG) (mg/ml)	1.5	0.45	1.2
Plasminogen		-	
Immunoglobulins (mg/ml)			
- IgA		-	
- IgG		-	
- IgM		-	
* HS = hepatitis-safe			

Tab. 5 Attributes of Factor VIII HS*
Comparison with normal Factor VIII concentrate and Factor VIII concentrate from another manufacturer

panzee experiments as regards the hepatitis safety of the processing, bearing in mind that under normal conditions patients with haemophilia have a seroconversion rate of practically 100% against HBV after the first injections of Factor VIII concentrates, and a clinical attack rate of at least 30%.

In the light of the experimental and clinical results it may be said that the possibility of transmission of hepatitis B by Factor VIII HS can be ruled out. Furthermore, non-A/non-B hepatitis has so far not been observed and the characteristic rises in transaminases and corresponding clinical signs have not been seen. However, long-term observation is being continued so that a definitive statement can be made.

During the course of clinical trial the following important indications for Factor VIII concentrate HS have crystallized:

- Young infants with severe haemophilia/initial treatment with Factor VIII concentrate.
- Traumatic haemorrhage or surgical operations in patients with mild or moderately severe haemophilia and in female carriers of haemophilia.

As regards the indications for Factor VIII HS it is worth noting that some children who already had signs of severe liver damage showed improvement during treatment with Factor VIII HS.

One of the most striking attributes of Factor VIII HS is its high purity – it is 450 times more

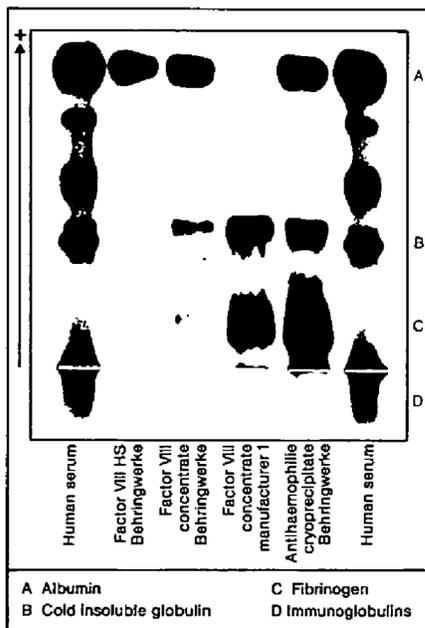


Fig. 3 Differences between various Factor VIII concentrates as revealed by screening electrophoresis

concentrated than plasma (Fig. 1) – and hence its low protein content of only 164 mg/1000 units Factor VIII. This gives a specific activity of 6.1 (Tab. 5). These values are even better than those for the commercially available non-heated Factor VIII concentrate, and this level is reached despite the need to stabilize Factor VIII HS by addition of 5 mg albumin/ml of the final solution. Its good solubility and, above all, its freedom from adverse effects are doubtless due

to the low protein content, in other words almost complete removal of immunoglobulins, of imperfectly soluble fibrinogen, cold-insoluble globulin (CIG) (Tab. 5).

Electrophoresis (Fig. 3) reveals that Factor VIII HS contains practically nothing at all from Factor VIII R:Ag and the albumin added as stabilizer. Furthermore, two-dimensional immunoelectrophoresis proves that the heating step does not cause denaturation of the Factor VIII molecule: there are no differences between it and the unheated molecule or the Factor VIII concentrate from another manufacturer¹³. The levels of Factor VIII-associated antigen and ristocetin cofactor (Tab. 5) also show that Factor VIII HS has been produced by a mild and non-traumatizing process.

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