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Factor VIII concentrates

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Discussion concerning Factor VIII and the treatment of haemophilia has recently reached an emotional level in the media and the question of cost has been raised as the sole criterion of medical activity. It can only be reduced to an objective scientific basis by means of precise laboratory and clinical studies. The standard of haemophilia treatment in the Federal Republic of Germany is among the highest in the world and is consequently dependent on high-quality Factor VIII concentrates, so as not to jeopardize all that has been achieved.

The discussion concerning Factor VIII in recent months has highlighted the question of the quality of the preparations on the market. Only clinical and experimental studies can provide the answer, taking account of the following criteria:

1. **Factor VIII activity** (standardization and testing)
2. **Protein content**
3. **Studies in patients** (in vivo recovery, half-life, side effects).

As Factor VIII concentrates are biological preparations which are isolated from human plasma the guidelines concerning standardization and purity that apply to synthetic drugs do not apply here. Each manufacturer uses his own fractionation method so the products are bound to vary; the Factor VIII preparations differ in several properties with the varying degree of purity being the most striking. With the preparations of some manufacturers, major deviations are found between different batches. The differences between high purity concentrates and less purified preparations are of an order of magnitude which, for clinical use, especially for massive therapy, must be clinically relevant and detectable in laboratory tests. Individual differences between patients are also possible, of course, and must be taken into account.

Standardization leaves questions open

As the Federal Health Office (BGA), which is the federal authority responsible for coagulation preparations, stipulates International Units according to the WHO standard for the approval of Factor VIII concentrates, the same requirements seem to apply for the standardization of all the Factor VIII concentrates at present on the market in Germany.

Binding test methods and batch testing similar to that carried out with comparable biological preparations do not exist however; thus there is as yet no objective standard for the Factor VIII activities given on the pack by the manufacturers.

Further problems are created by the fact that we, as an individual manufacturer with a correspondingly large fractionation capacity, manufacture Factor VIII concentrates in Germany and also market them here. All other Factor VIII concentrates that are on the market in Germany are produced abroad and imported. This latter amount accounts for 90% of total consumption, most of it coming from the USA.

The standard applicable in the USA for Factor VIII is the BOB standard (Bureau of Biologicals) of the FDA (Food and Drug Administration). This FDA standard is 20% lower than that of the WHO; i.e. 1 WHO unit corresponds to 0.8 FDA units (1).

In investigations carried out in the USA into the three leading Factor VIII concentrates on the market two preparations were found to have only 50% and one preparation 75% of the Factor VIII activity given on the pack, based on WHO units (2). The in vivo recoveries correlated with these results and for the first two preparations were in the region of 60%, and for the third preparations was in the expected range of 100%.

Similar results were obtained in an English study (3) which, for one manufacturer, determined an underfilling of 20%, averaged out over several batches. The authors, like those in the USA, come to the conclusion that the dosage for effective substitution therapy – based on the declared units – had to be considerably raised for these preparations and therefore a preparation which seems cheap at first sight proves more expensive over the entire period of therapy (2, 3, 4).

A comparative study carried out in Germany with three Factor VIII concentrates showed that the preparation of an American manufacturer contained considerably fewer units than were declared on the pack (5).

Clearly, the results of these studies compelled two American manufacturers to fill to a higher standard in 1981, at least in the case of the preparations they market in Germany.

As, since the middle of last year, various parallel importers have been buying up original US goods in the USA and marketing them in Germany, it is possible that BOB-standardized Factor VIII concentrates are being used with an underfilling of about 20%, compared with the WHO standard.

As a further complicating factor in filling there is the fact that the testing of concentrates is not without its problems and only yields reliable and reproducible results in experienced laboratories. A margin of error of ± 20% is possible with individual determinations (3). There are also differing opinions as to the reproducibility and clinical relevance of the results of the one-phase and two-phase tests, with the two-phase test being favoured in England.

Simultaneous trials of Factor VIII concentrates with the one-phase and two-phase tests give rise to varying results: Whereas a French study group always found higher values with a two-phase test (+ 14%) (7), the second part of the German study (6) shows consistently lower values for the two-phase test.

Protein-chemical properties of Factor VIII concentrates

As there is no batch approval system for Factor VIII concentrate on the part of the monitoring authorities in Germany, the doctor treating haemophilia is largely dependent on the information given by the manufacturer. This applies in particular to the Factor VIII activity on the basis of which the dose is decided, and to an increasing extent, for the protein composition of the preparations on which information can only be obtained by carrying out our own trials. It is asking too much even of large centres with the necessary laboratory equipment and personnel to determine those parameters that are important for therapy as a matter of routine with each batch.

Comparative trials of Factor VIII concentrates thus assume greater importance. Very recent examples have shown that the publications of such trials (2, 5, 6) is quite capable of drawing attention to qualitative defects and effecting improvements.

We have already published the results of such trials and have compared our new developments Factor VIII concentrate and Factor VIII – hepatitis-free – with important preparations on the market. It was possible to show that much of the innovation in the development of Factor VIII concentrates, and thus the treatment of haemophilia patients, was based on our research. In the mid-70s a fibrinogen-free Factor VIII high concentrate was produced for the first time on a large scale (8) and improved in the years that followed (9). In 1980 we succeeded in manufacturing the first hepatitis-free Factor VIII concentrate for therapy in the world (9, 10) – a logical development for biochemical research, whose aim it should be to isolate the factor lacking in the patient in the most unchanged form possible, free from foreign proteins and infectious material, and make it available for subsitution therapy.

The fact that in the meantime two other firms have developed a highly purified preparation and others are trying to produce a hepatitis-free Factor VIII concentrate emphasizes that our endeavours are setting a trend.

Extensive in vitro and in vivo studies of Factor VIII concentrates have also been published by French (7), Swedish (11) and Austrian (12) groups, taking account of preparations which play an important role in these countries alone.

In an initial series of trials early in 1981 we investigated the most important Factor VIII concentrates at present on the market in Germany. These were in each case a filling of one batch from 6 manufacturers and a marketing company; in the case of one manufacturer a filling of a second batch was also investigated.

New preparations from American manufacturers and parallel-imported Factor VIII concentrates mainly from the USA which came on to the market in Germany in the second half of 1981 inspired us to carry out a second series of trials. Individually, the products consisted of a new approved form, a product from a change in manufacturing procedure and parallel imports of two American preparations. Again one filling from each batch was investigated in each case. All the preparations were purchased directly from the market. The Behring preparations that were investigated at the same time were removed blind from current production. At the same time the most important analytical results of all the batches manufactured in Behringwerke in 1981 were evaluated.

Material and methods

Material

Factor VIII concentrate and Factor VIII HS – Behringwerke; Factor VIII concentrates from 8 other manufacturers. Glycine, saccharose, common salt, trisodium citrate, all in p.a. quality from E. Merck, Darmstadt.

Aluminium hydroxide suspension, 1% w/v from British Drug Houses (U. K.).

Pathromtin®, coagulation factor VIII-deficient plasma, Multifibren®, Tri-Partigen®-IgA, -IgG, -IgM, M-Partigen®-Fibrinogen, LC-Partigen®-Fibronectin from Behringwerke AG, Marburg/Lahn.

Methods

Determination of Factor VIII coagulation activity

(F VIII:C): One-phase method according to Simone et al. (13) with the reagents of Behringwerke AG, Marburg/Lahn. Information in I.U. according to WHO standard or Substandard: deep-frozen, pooled citrated human plasma from male donors.

Factor VIII R: Ag: Electroimmunodiffusion after Laurell (14): Evaluation by means of a reference curve produced with dilutions of fresh plasma. Details of concentration and activity given in units on the assumption that 1 ml of fresh citrated mixed plasma contains 1 U of Factor VIII-associated antigen (F VIII R:Ag).

Protein determination: Method according to Lowry et al. (15).

Determination of plasma proteins: Function test of fibrinogen after Clauss (16).

IgG, IgM, IgA, fibrinogen and fibronectin on Tri-, M- and LC-Partigen® immunodiffusion plates from Behringwerke according to the principle of radial immunodiffusion after Mancini et al. (17).

Screening electrophoresis after Laurell (14): In 0.8% agarose and sodium diethyl barbiturate acetate buffer 0.1 mol/l, pH 8.2, running time 8 h, field strength 1.5 V/cm; staining with amidoblack. Of the Factor VIII preparations in each case 25 U F VIII:C/ml was applied; reference substances: fibronectin (CIG), fibrinogen and immunoglobulin (IgG) as 0.5% solutions.

Immunoelectrophoresis on agarose plates in the micromodification of Schwick and Störiko (18).

Results

Results of the first series of trials

The Factor VIII activity in our preparations is determined with the aid of a one-phase test (13) and adjusted in WHO units by means of a plasma standard calibrated against the WHO

International Standard 2. The activities of all the preparations were investigated by means of this test system.

Comparison of the declared activity of the different manufacturers with that actually found shows deviations of + 20% to −40% (Tab. 1). An underfilling against the WHO standard is therefore also evident in this study especially with some American preparations, though the differences between the various preparations are not as marked as they were in the first study by Schimpf (5).

At 3.5, the **specific activity** (Factor VIII activity per mg protein) of our Factor VIII concentrate is far higher than that of any other preparation. This value is confirmed by Vukovich (12) at 3.4. In addition it should be taken into account that, before filling, we add albumin in a final concentration of 5 mg/ml for stabilization; thus if this amount of albumin is removed there is a specific activity of 10.

Preparation 2, a highly purified preparation developed in the USA is next in degree of purity with a specific activity of 2.2. followed by concentrate 3 which has already been on the market for years and has a specific activity of 1.7. All other Factor VIII preparations have a specific activity of less than 1 and must therefore be termed preparations of moderate purity.

The **ratio F VIII R:Ag/F VIII:C** enables conclusions to be drawn as to how gentle a manufacturing method is as well as to the quality of the starting plasma. Here a ratio of 2:1 should be described as good as this value is already obtained in the cryoprecipitate (12). Some preparations – 4, 5a, 8, 3 – are considerably higher with values of between 3.5 and 4.9.

Furthermore, Table 1 illustrates the total protein content **of the Factor VIII concentrates** and the distribution over **the main protein classes.** All the values were calculated per 1000 U F VIII:C because in clinical application this reference allows an objective comparison of the quantities of protein in the preparation approximating to bulk practice. The Factor VIII concentration in the plasma is only about 1 mg/100 ml. When considering the total protein therefore Factor VIII is only a minor component in terms of weight. The total protein consists largely of associated proteins which are carried over into the end product by the various manufacturing methods. The Behring preparation is an exception here: Our manufacturing method (9) produces a largely proteinfree Factor VIII product which contains, in addition to small amounts of CIG (cold insoluble globulin = fibronectin) only a few traces of other proteins. 0.5% albumin is added for stabilization.

Albumin therefore accounts for two-thirds of the amount of protein of 288 mg. When considering the total protein it is necessary to decide whether it consists of foreign proteins which originate from the manufacturing method and have not been removed during fractionation or whether it is albumin that has been subsequently added. This difference becomes evident when considering preparations 2 and 3, which with 460 and 584 mg total protein respectively come next to our preparation but – as is shown by screening and immunoelectrophoresis – contain no detectable albumin or only traces of it. The impurities are therefore due to other proteins such as fibrinogen, fibronectin and immunoglobulins.

The **protein contents of the preparations of moderate purity** 4, 5, 7, 8 are between 1.1 and 2.3 g/1000 U. Consideration of the differentiation of the individual proteins shows that our Factor VIII concentrate is, according to the declaration, free from coagulable fibrinogen and contains only small amounts of immunologically detectable fibrinogen. The range of concentrations with the other preparations extends from 324 mg to 1.3 g fibrinogen and this, at over half the total protein, constitutes the majority of the impurity. Our preparation contains only small amounts of CIG (58 mg) as the only foreign protein; here Preparation 2 is somewhat better with 13 mg. The other preparations contain considerably higher amounts of CIG.

Immunoglobulins are present in our preparation only in traces and are

Tab. 1: Activity and protein-chemical characterization of Factor VIII concentrates (1st series of trials)

Preparation	Determination of F VIII:C and F VIII R:Ag				Relationship F VIII R:Ag/F VIII:C	Specific activity (UF VIII:C/mg (protein)	Protein content (mg/1000 U)	Immunoglobulin content (mg/1000 U)			Fibrinogen content (mg/1000 U)		Fibronectin (CIG) (mg/1000 U)
	F VIII:C (U/fillg) declared	determined	Deviation %	F VIII R:Ag (U/1000 U F VIII:C)				IgA	IgG	IgM	functional test (Clauss)	immunological determination (M-Partigen)	
1*	1000	1200	+20	2033	2.0	3.5***	288	–	–	–	–	14	58
2	1000	840	−16	1893	1.9	2.2	460	–	18	8	370	436	13
3	250	250	± 0	3480	3.5	1.7	584	–	48	16	304	324	288
4	270	160	−40	4875	4.9	0.7	1550	31	163	19	900	1175	325
5	500	540	+ 8	2778	2.8	0.7	1354	30	141	19	533	691	69
5a	250	180	−28	4500	4.5	0.6	1728	39	211	22	1017	1311	295
6**	500	600	+20	2400	2.4	1.8	567	15	78	7	305	363	192
7	250	270	+ 8	2444	2.4	0.9	1122	15	107	7	533	678	141
8	250	180	−28	3778	3.8	0.4	2356	50	239	22	767	1017	211

* Factor VIII conc. Behringwerke batch 433311
** Marketing company
*** without albumin there is a specific activity of 10

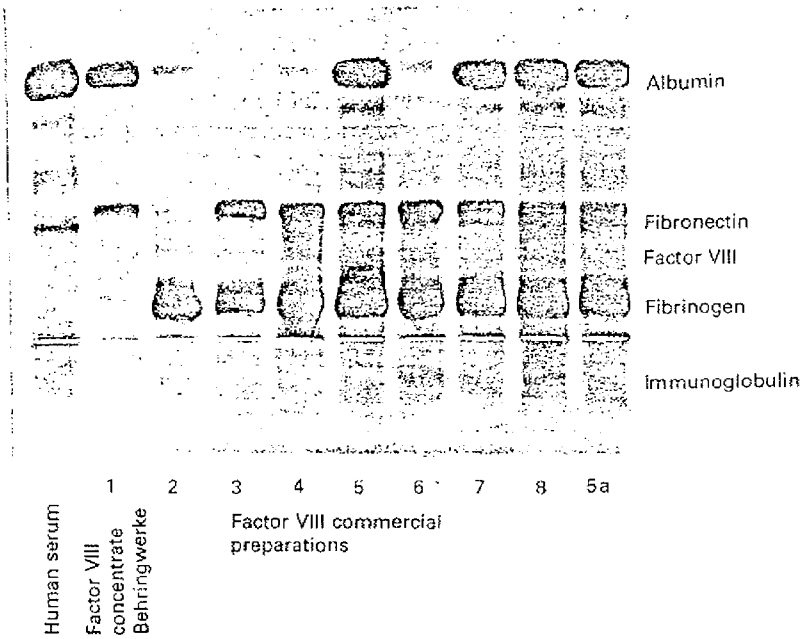

Fig. 1: Characterization of various Factor VIII concentrates in screening electrophoresis.

below the detection limit. Preparation 2 and to an even greater extent preparation 3 have low immunoglobulin values, whereas all the others contain 200 to 300 mg immunoglobulins of all three classes per 1000 U.

Screening electrophoresis (Fig. 1) impressively demonstrates the variety in protein composition of the Factor VIII concentrates and confirms the quantitative findings. Factor VIII concentrate from Behringwerke has a marked albumin and a weak CIG band; fibrinogen and immunoglobulins are below the detection limit. Preparation 2 shows a pronounced fibrinogen band. Preparation 3 possesses marked fibrinogen, CIG and immunoglobulin bands which are even more pronounced in preparation 4.

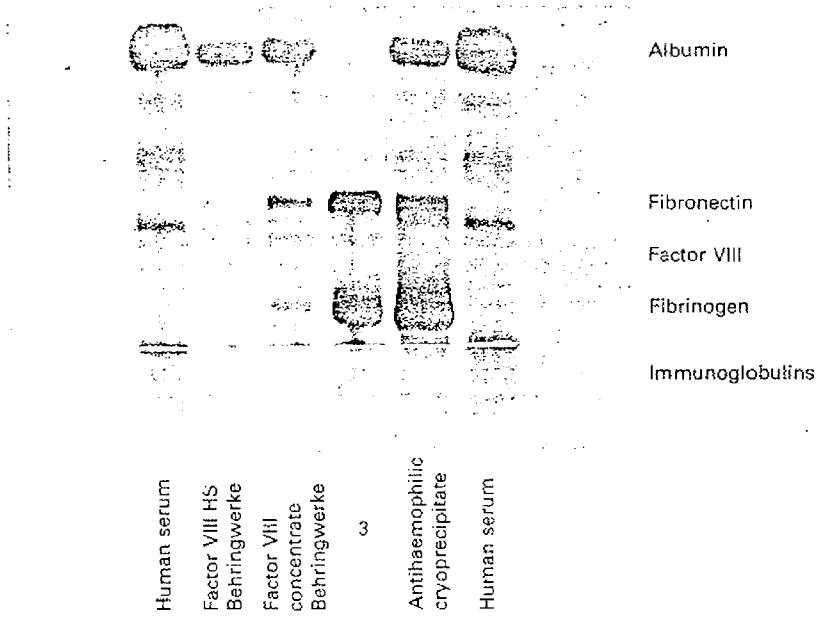

Fig. 2: Characterization of various Factor VIII concentrates in screening electrophoresis.

All other Factor VIII preparations demonstrate the same protein pattern as cryoprecipitate (Fig. 2).

Immunoelectrophoresis (Fig. 3) leads qualitatively to the same results, with the immunoglobulin bands clearly visible in all preparations apart from the Behring product, and additional protein bands detectable with the preparations of moderate purity.

In summary, it was found for the preparations of the first series of trials, which are all on the market, that the division into high purity concentrates and preparations of moderate purity, which were selected according to their specific activity is fully confirmed by consideration of the protein composition of the different preparations.

Results of the second series of trials

The second series of trials were intended to indicate whether those Factor VIII concentrates parallel-imported from the USA in the second half of 1981 differ qualitatively from the preparations already on the market in Germany. We shall not go into details here of the legal problems and inadequacies in labelling of the packs of parallel-imported goods in Germany.

In determining Factor VIII activity it was necessary to test to what extent the parallel-imported goods from the USA are filled according to the BOB standard which is applicable there. This is clearly necessary in the case of preparation 3a, for which we found a deviation of −27% from the declared units.

Schimpf (6) determined a deviation of similar order of magnitude. The two other batches of preparation 4, parallel-imported by various marketing companies demonstrated no negative deviations in activity (Table 2).

In the other aspects, too, the parallel-imported preparation 3a was so poor that it has to be designated a preparation of moderate purity. We have investigated the preparations of this manufacturer on a number of occasions (8, 9) (Tab. 1) but have always found better results. Thus the specific activity of 0.7, the protein content of over 1.5 g, the fibrinogen of 585 mg and the high immunoglobulin values are two to three times higher

than those otherwise found for this product. The two parallel-imported batches of preparation 4 investigated by us show differences in the **specific activity** 1.2 and 1.3 to 1.5, and therefore in the protein content, from the directly marketed preparation; these are however considerably less marked than for preparation 3. Preparation 4 is – when one considers the protein and immunoglobulin content – a preparation of medium purity; however the values in the second series of tests are slightly better than in the first. With preparation 2 the results of the first test series are largely confirmed. A newly approved preparation 9, which is also produced in the USA, represents, in terms of its composition, no enrichment of the Factor VIII concentrates. With a specific activity of 0.7 and a protein content of almost 1.5 g/1000 U it bridges the gap between preparations of moderate purity 4, 5, 7. The greatest advance in the degree of purity was achieved with preparation 8, which was transformed by a new manufacturing method into a high purity concentrate with a specific activity of 7. Figures 4 and 5 show that the preparation contains no albumin

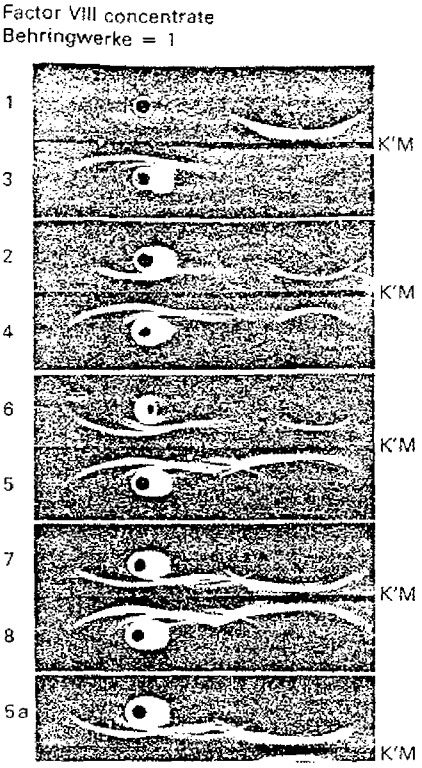

Fig. 3: Characterization of Factor VIII concentrates in immunoelectrophoresis (against anti-human serum from rabbits, K'M).

and that total protein of 144 mg is therefore composed largely of immunoglobulins, fibrinogen and CIG. The two factor VIII concentrates of Behringwerke, Factor VIII concentrate and the more highly purified Factor VIII HS, with 293 and 196 mg total protein respectively are presented in direct contrast to these; two-thirds of these amounts are due to albumin that has been added subsequently however.

The high degree of purity of our preparations and of preparation 8 is apparent from the dissolution time and the appearance of the solution. The preparations dissolved very rapidly to give colourless clear to faintly opalescent solutions; the same applies to preparation 2 within certain limitations. The less purified preparations of the second series required a longer dissolution time however and sometimes yielded yellow cloudy solutions.

The determinations of Factor VIII R:Ag produced values in the second series which were considerably higher than those obtained in the first series, probably as a result of the method used. The comparison of the relative

Tab. 2: Activity and protein-chemical characterization of Factor VIII concentrates (2nd series of trials)

Preparation	Solution volume (ml)	Determination of F VIII:C and F VIII R:Ag				Relationship F VIII R:Ag/F VIII:C	Specific activity (UF VIII:C/mg protein)	Protein content (mg/1000 U)	Immunoglobulin content (mg/1000 U)			Fibrinogen content (mg/1000 U)	
		F VIII:C (U/fillg) declared	determined	Deviation %	F VIII R:Ag (U/1000 U F VIII:C)				IgA	IgG	IgM	functional test (Clauss)	immunological determination (M-Partigen)
3a (parallel-imported by A)	20	518	380	−27	6474	6.5	0.7	1542.1	4.2	73.7	17.9	585.3	717.4
2	10	250	250	± 0	5480	5.5	2.9	348.0	2.8	18	9.2	281.4	394.0
4	10	270	280	+ 4	4393	4.4	1.5	685.7	14.3	91.1	11.4	221.8	417.9
4a (parallel-imported by A)	10	220	220	± 0	6273	6.3	1.3	790.1	15.5	104.5	13.7	252.7	431.8
4b (parallel-imported by B)	40	1060	1120	+ 6	5464	5.5	1.2	810.7	17.14	94.4	12.9	251.4	410.7
9	20	500	540	+ 8	5963	6.0	0.7	1466.7	20.4	99.3	12.6	521.5	629.6
8	10	310	390	+26	3538	3.5	7	143.6	1.8	51.3	4.1	20.5	37.2
Factor VIII conc. Behringwerke Batch 433325	40	1000	1120	+12	3714	3.7	3.4***	292.9	0.5	4.6	1.9	–	44.3
Factor VIII HS Behringwerke Batch 439062	20	500	560	+12	4714	4.7	5.1***	196.4	0.3	–	1.2	–	16.1

*** see Tab. 1.

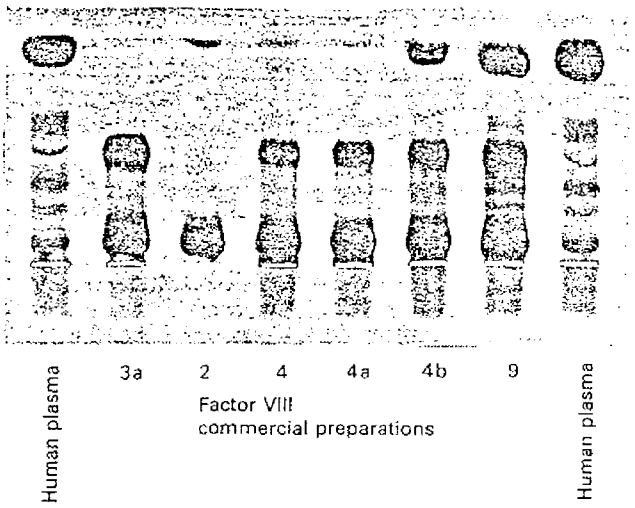

Fig. 4: Characterization of various Factor VIII concentrates in screening electrophoresis.

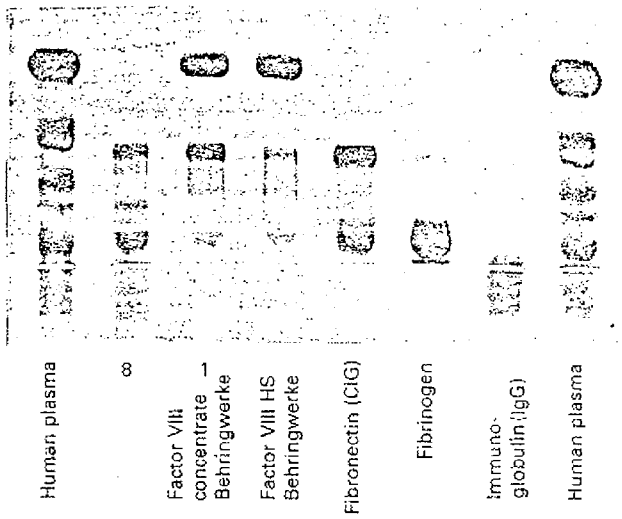

Fig. 5: Characterization of various Factor VIII concentrates in screening electrophoresis (fibronectin, fibrinogen and immunoglobulin are applied as reference substances).

proportions in the preparations with each other remains unaffected however.

The **results of the quantitative studies** are visually confirmed by screening electrophoresis (Figs. 4 and 5). With the highly concentrated products the protein pattern on screening electrophoresis is so simple and characteristic for the preparations that they can be identified by means of their electrophoretograms, which are comparable to finger prints.

The studies show that the differences in quality of the Factor VIII concentrates at present on the market in Germany are very wide-ranging: total protein of 150 to 1500 mg, fibrinogen of 10 to 1000 mg and IgG 10 to 200 mg/1000 U. It is therefore inevitable that, due to this 10–100 times higher level of total protein or of individual proteins in the less purified preparations, the patient may experience undesirable reactions, whatever form these may take.

The road we have embarked upon involving the extensive purification of Factor VIII has since been taken by other manufacturers. For high purity concentrates the limit for specific activity should be set at 3. Because of the different test systems and standards of the various manufacturers, marked variations from the declared values are sometimes detectable in the Factor VIII activity of the different preparations, although in 1981 there was clearly a somewhat higher filling, especially in the case of American preparations.

With some manufacturers marked qualitative differences between various batches are sometimes to be found. It is also possible that preparations with lower activity and a higher protein content are coming on to the market as parallel-imported goods.

A survey given in Table 3 of all the 54 batches of Factor VIII concentrate and 44 batches of Factor VIII HS produced in 1981 shows for Factor VIII activity an average value of 30.11 and 29.68 U/ml; the standard deviation is in the region of 4.45 and 4.95 units.

As 25 units/ml are declared, this more or less ensures – taking account of a margin of error for Factor VIII testing of about 20% – that the pack contains at least the declared amount of Factor VIII. Similarly the values for total protein, at 8.58 and 7.79 with standard deviations of 1.75 and 1.21, show a high degree of consistency in the composition of the product.

Further progress in the field of Factor VIII, also in terms of less costly production, can be achieved by stepping up our research activities. Only then can the new technologies evolving in protein chemistry be included.

Tab. 3: Average values of Factor VIII activity and total protein of all the batches of Factor VIII concentrate and Factor VIII-HS manufactured at Behringwerke in 1981

	Number of batches	Factor VIII activity F VIII:C (U/ml)	Total protein* (mg/ml)
Factor VIII concentrate	54	$\bar{x}$ = 30.11 (s = 4.45)	$\bar{x}$ = 8.58 (s = 1.75)
Factor VIII-HS Behringwerke	44	$\bar{x}$ = 29.68 (s = 4.95)	$\bar{x}$ = 7.79 (s = 1.21)

* this includes about 5 mg/ml albumin, which is added for stabilization

Summary

For effective haemophilia treatment high quality preparations are needed. This means standardized, highly purified Factor VIII concentrates, free from infectious material, particularly hepatitis viruses. To resolve the question of quality, we

carried out two series of trials – at the beginning and end of 1981 – to investigate all the Factor VIII preparations on the market in Germany for Factor VIII activity and protein-chemical composition. The results show that the range of protein concentrations is wide: total protein of 150–1500 mg, fibrinogen 10–1000 mg and IgG 10–200 mg/1000 units of Factor VIII. The differences between high purity concentrates and less purified preparations are of orders of magnitude which, for clinical application, especially with massive therapy, must be clinically relevant and detectable in laboratory tests. Similarly, with Factor VIII activity marked negative deviations from the declared values are sometimes to be found. The road to extensive purification of Factor VIII embarked on by Behringwerke has since been taken by other manufacturers. The limit for specific activity should be set at a value of 3 for high-purity concentrates. In view of the results obtained it is evident that preparations with lower activity and a higher protein content are coming on to the market, and therefore into use, as parallel imports.

We are grateful to Professor Norbert Heimburger for his valuable help in carrying out this work.

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Übersetzung