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WORKSHOP ON POTENCY AND DOSAGE OF von WILLEBRAND FACTOR CONCENTRATES

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1 P R O C E E D I N G S

2 **Welcome and Opening Remarks**

3 DR. FEIGAL: Good morning. My name is David
4 Feigal. I am the Deputy Director of the Center for
5 Biologics. It is my pleasure to welcome you all here this
6 morning.

7 One of the things that I thought I would just
8 begin with is what is the role of FDA in the regulation of
9 blood products. If you look at the history of the standards
10 that have been set, they come from two sources: One comes
11 from the Public Health Act and the other from the Food, Drug
12 and Cosmetic Act. They do a great deal to define our role
13 and what the standards are, and it has a lot to do with the
14 business that we have framed today.

15 Some of the things that we do not, we do not
16 regulate the practice of medicine, and that includes off-
17 label uses of approved products. We also do not control the
18 use of pharmacies, or have any role in setting prices or
19 purchasing in federal drug programs or drug availability
20 programs. But, indirectly, I think we obviously have an
21 important effect on all of those.

22 The key words in the Public Health Service Act
23 about the products that we regulate are that we are charged
24 by Congress to assure that they are safe, pure and potent.

1 The potency standard, actually, predated the efficacy
2 standard for drugs. Biological products had to demonstrate
3 that they worked long before Congress demanded that we know
4 that drugs work. Part of the issue today is the issue of
5 how do we define potency, and what are the issues that
6 relate to establishing the relationship between potency and
7 effectiveness for this group of products.
8 Because these are products which make claims that
9 are defined in the Food, Drug and Cosmetic Act, claims for
10 products which ameliorate disease, that Act also defines the
11 nature of the standards of where we collect evidence from
12 for FDA approval, and that is from controlled clinical
13 trials. Controlled clinical trials, as described even in
14 the regulations, is not simply the highest standard,
15 placebo-controlled trials, but it also includes trials which
16 show dose responses; trials which compare one agent to
17 another; and trials which compare to no treatment or even
18 historical controls. All of these are sources of evidence.
19 One of the things that I think is best known,
20 particularly in the blood area, of what FDA's role often
21 becomes is setting standards for products that are made
22 available by multiple manufacturers so that when you read
23 the labeling for a product, and it describes a set of
24 properties, there is some consistency and there are some

1 standards that determine what those properties are.

2 This is a disease area and a product area that has
3 challenges in all these areas, and we are grateful that this
4 is a cooperative effort with the National Heart, Lung and
5 Blood Institute, that we have participation from other
6 regulatory authorities, from the academic community and,
7 importantly, from people who use these products so that we
8 can see if there is a way that we can define standards,
9 collectively describe the evidence and make this a set of
10 products that are more straightforward for use.

11 So, I welcome you and thank you very much for your
12 participation today.

13 **Session I: Regulatory Perspective on**
14 **Licensure of vWF Concentrates**

15 **FDA Perspective: von Willebrand Factor**

16 DR. WEINSTEIN: Thank you very much, Dr. Feigal.
17 I am Mark Weinstein. I am Director of the Division of
18 Hematology, here, at CBER. Before we start the formal part
19 of this meeting, I would like to express my thanks to a
20 number of people who have helped to make this meeting
21 possible. These include Andrew Chang, who did much of the
22 work in arranging the scheduling of speakers; my colleague
23 and co-chairman, Margaret Rick, from the NIH; and the staff
24 at CBER, particularly Jo Wilczek, who arranged for the

1 advertising and promotion of this meeting. I would also
2 like to thank the speakers who have taken so much time and
3 effort in arranging their presentations for this conference.
4 Last, but hardly least, I would like to thank you, the
5 audience, for coming and being involved in this meeting and
6 being active participants in bringing issues forward here.
7 The success of this meeting will depend a great deal on your
8 active participation, and commenting on the presentations
9 and bringing issues of concern forward that you might have.
10 I will next briefly review the agenda of this
11 Workshop, which is devoted to the issue of potency and
12 dosage of von Willebrand factor concentrates.
13 In the first part of this meeting we will discuss
14 von Willebrand factor from a regulatory perspective, as seen
15 as the vantage point of the U.S. regulatory agency and from
16 the perspective of European regulatory agencies, and
17 particularly the question about how can this product be
18 available in Europe, license in Europe for use of von
19 Willebrand factor indications, and use in the United States.
20 Is this a uniform situation in Europe, and is there
21 something different about their regulations as compared to
22 ours?
23 Next we will have a presentation from a consumer
24 representative, who will tell about her experiences as a

1 user of von Willebrand factor concentrates. This will be
2 followed by presentations from clinicians who will talk bout
3 their perspectives, practices and expectations. We wish to
4 explore the range of options, procedures and what
5 information do clinicians desire to use this product
6 properly. In this regard, I particularly want to thank Drs.
7 Jeanne Lusher and Alice Cohen who went to the trouble of
8 doing survey work in this area particularly and specifically
9 for this meeting. I feel that these surveys will help us
10 get a broad perspective of how these products are used.
11 The next part of the meeting will consist of
12 presentations about various assays of von Willebrand factor
13 in an effort to get a better understanding of the status of
14 measurements of von Willebrand factor functional activity.
15 Can assays be selected that will correlate with clinical
16 benefit, or at least provide critical characteristics of
17 these products that can be used to define and standardize
18 von Willebrand factor products?
19 The manufacturers will then have an opportunity to
20 discuss investigations that they have conducted on the
21 materials that contain von Willebrand factor. We should get
22 a sense of the range of properties of these materials and
23 what tests have been used to characterize them. Of
24 particular interest will be the experiences that

1 manufacturers have had in performing clinical trials with
2 these products. We will get a sense of the difficulties
3 that they have had, the kinds of assays that have been used,
4 and perhaps derive from this a better sense of what is a
5 practical kind of clinical trial that we can use to get
6 these products licensed.

7 In the last part of the meeting we will have a
8 panel discussion where we will talk about the previous
9 presentations and attempt to answer questions, such as what
10 in vitro laboratory measurements best reflect von Willebrand
11 factor activity in vivo, and how should doses be selected
12 for study in clinical trials of von Willebrand factor
13 concentrate. There should also be time throughout the
14 meeting for your active participation in this process.

15 I will first start out by discussing very briefly
16 von Willebrand factor from the perspective of the FDA. This
17 will be expanded upon in the next talk with my colleague,
18 Dr. Ross Pierce, from the FDA.

19 (Slide)

20 This is the title of the presentation.

21 (Slide)

22 The present situation in this country is that
23 there is no product licensed for the indication of von
24 Willebrand factor. In the United States patients are

1 treated with Factor VIII concentrates that contain von
2 Willebrand factor but lack labeling for the von Willebrand
3 factor content or dosage.
4 (Slide)
5 This has led, of course, to a number of difficult
6 problems. Products do not have the von Willebrand factor
7 content on the label and, therefore, there is potential for
8 mishandling. We know that we have gotten complaints from
9 physicians who have been in a situation of ordering Factor
10 VIII from the pharmacy to treat a von Willebrand factor-
11 deficient patient and receiving material that contained, in
12 fact, Factor VIII with very little von Willebrand factor
13 present and found, of course, that the products were
14 useless. So there is a definite need for having a content
15 of von Willebrand factor on products that contain the
16 material, and that can be used for the treatment of von
17 Willebrand factor disease.
18 Another problem is that the dosing is based on
19 Factor VIII in many cases rather than von Willebrand factor,
20 which is, of course, the defect of protein. This works out
21 okay in many cases and people can get along with that for
22 some products, but for other products, particularly some
23 that do not contain any Factor VIII coagulant protein at
24 all, this dosing problem can be very difficult. How do you

1 handle the dosing of that kind of product? We will be
2 hearing today from some folks from France who do use
3 products of this nature, and it will be interesting to hear
4 how this is accomplished.
5 There is also a consensus within the community
6 that says that ristocetin cofactor activity is adequate as a
7 functional assay. Clinicians could make informed treatment
8 regimens based on von Willebrand factor content. Later on
9 we will explore whether this statement is, in fact, in true.
10 Is this a good way of defining what the content of von
11 Willebrand factor activity is?
12 (Slide)
13 As Dr. Feigal pointed out, we are constrained at
14 the FDA by the Code of Federal Regulations to follow these
15 definitions for getting products approved. There is a
16 necessity of having a definition or potency label, that is,
17 the specific ability or capacity of the product, as
18 indicated by appropriate laboratory tests or by adequately
19 controlled clinical data obtained through administration of
20 the product in a manner intended to effect the given result.
21 So, in order to have a produce licensed, it has to have some
22 definition of potency on it. How you do that for von
23 Willebrand factor when we don't have adequate standards or a
24 definition, in fact, of what von Willebrand factor activity

1 is?

2 Secondly, there has to be information about

3 dosage. Labeling shall state the recommended usual dose,

4 the usual dosage range. Dosages shall be stated for each

5 indication when appropriate. This section of the label

6 shall also state the intervals recommended between doses,

7 the optimal method of titrating dosage, and the usual

8 duration of treatment. Many of the discussions that we will

9 have later on today will address how we should devise

10 studies to assess what the proper dosage of von Willebrand

11 factor should be.

12 (Slide)

13 Also, in the complex language of the FDA and the

14 CFR, the labeling of a drug may be considered to be

15 misleading by reason of failure to reveal the proportion of,

16 other fact, with respect to an ingredient present in such

17 drug when such proportion or other fact is material in the

18 light of the representation that such ingredient is present

19 in such drug.

20 This needs a little translation. This means that

21 one has to be able to say how much of something is in a drug

22 if you say that it is going to have some degree of

23 effectiveness, that it is going to be used for a certain

24 treatment. Of course, with von Willebrand factor, where you

1 don't have a definition of what the element is of potency,
2 you have a dilemma in how you are going to define what the
3 product is. So, we have to get over this notion here of
4 what we should call, what the measurement should be of
5 Factor VIII, what it should say on the label, how many units
6 of von Willebrand factor are present in the product.
7 (Slide)
8 Future directions that I hope will be explored in
9 this meeting are new assays for von Willebrand factor, such
10 as the collagen binding assay, shear-induced platelet
11 aggregation. Maybe, with our present state of knowledge,
12 the ristocetin cofactor activity is sufficient for defining
13 the activity of the protein. Are new clinical studies
14 needed to better correlate the von Willebrand factor
15 property with clinical outcome? Should there be a new von
16 Willebrand factor concentrate standard? These are all
17 issues that will come forward, I hope, in our discussions
18 and that will lead to licensure of these products in the
19 near future.
20 I would next like to turn this over to Dr. Ross
21 Pierce, from the Division of Blood Applications, who will
22 further define what the requirements are for the FDA
23 licensure of these products. Thank you.
24 **Evaluation of vWF Concentrates and**

1 **FDA Reviewers' Approach**

2 DR. PIERCE: Thanks very much, Mark.

3 (Slide)

4 First I am going to discuss the type of approach
5 that we would take in general for biologic products at FDA.
6 Among the things we consider are evaluation of product
7 potency. We ask the questions which in vitro assay is most
8 appropriate as a reliable indicator of clinical activity?
9 Does the manufacturing method assure lot-to-lot consistency
10 of potency and safety? How should studies be designed in
11 order to determine the correlation between in vitro potency
12 and efficacy in the clinic? Which are the appropriate
13 clinical settings for determining the efficacy and safety of
14 the product? And, is sufficient information available to
15 make informed dosing recommendations? Pharmacokinetic
16 studies can help us with the last point but do not provide
17 the whole answer.

18 (Slide)

19 Ristocetin cofactor activity has been proposed as
20 a measure of potency but this assay does not provide a
21 perfect solution at the present time. As is shown on this
22 slide, from a published FDA study, different laboratories
23 can obtain results that differ by as much as 75% relatively
24 when measuring this activity on the same sample. Also,

1 correlation of ristocetin cofactor activity with correction
2 of bleeding time has been poor, but we know that the
3 correlation of bleeding time correction and clinical
4 hemostasis also is certainly less than perfect.
5 (Slide)
6 Here we see that different von Willebrand factor
7 concentrate products demonstrate markedly different ratios
8 of ristocetin cofactor activity to Factor VIII activity
9 level, with some products essentially devoid of Factor VIII.
10 (Slide)
11 As an interim measure, absent a valid single _in
12 vitro_ measurement to assure product potency and consistency,
13 FDA might consider using a combination of assays such as
14 ristocetin cofactor activity, Factor VIII in those products
15 that contain it, multimer pattern and von Willebrand factor
16 antigen.
17 (Slide)
18 What we have asked sponsors of these concentrated
19 products, who are interested in pursuing a von Willebrand
20 factor disease indication, to do is to study the product's
21 pharmacokinetics, to perform clinical studies relevant to
22 the proposed use and to document consistency in
23 manufacturing.
24 (Slide)

1 A good pharmacokinetic study in this disease
2 should give us the following: the in vivo recovery, a handle
3 on the variability within and between patients in in vivo
4 recovery, plasma elimination half-life, the variability in
5 that half-life, and we also consider it desirable to
6 understand the influence, if any, of disease subtype
7 severity and the severity of the bleeding episode on the
8 half-life.
9 (Slide)
10 So, whom should we study in clinical trials that
11 concern efficacy and safety? Well, subjects who have a
12 documented history of abnormal bleeding episodes are
13 certainly relevant and important. Subjects who would be
14 likely to receive the product in actual practice -- this
15 would exclude mild type 1 patients who may be managed
16 satisfactorily with alternative agents.
17 We also need to recognize that if we restrict
18 enrollment to the most severely affected patients, such as
19 type 3, that will limit our study size and power due to the
20 rarity of these patients.
21 (Slide)
22 We also need to ask the question what kinds of
23 evidence do we need to say a product works in different
24 settings, namely, surgery, the treatment of spontaneous

1 bleeding and the prophylactic use in the prevention of
2 spontaneous bleeding.
3 (Slide)
4 We also need to consider the question of which
5 clinical endpoints are most relevant to understanding
6 product efficacy. Satisfactory clinical endpoints should
7 reflect disease severity in the untreated condition
8 distinguishing patients from normal individuals, of course.
9 One hopes that they demonstrate minimal inter- and intra-
10 observer measurement variability and, preferably, they
11 should be objective in order to minimize bias. Examples of
12 objective endpoints would include duration of spontaneous
13 bleeding or surgical wound oozing and estimated
14 perioperative blood loss in number of units lost or
15 replaced.
16 (Slide)
17 Subjective endpoints are another possible choice
18 but do present some problems. A subjective global
19 assessment of bleeding could be a dichotomous variable,
20 normalized or not normalized bleeding tendency. It could be
21 a scale of quantities, such as three or more categories like
22 excellent, good, fair, poor, abysmal. However, subjective
23 endpoints can be associated with significant variability
24 and, if true, that can limit power and reliability, and

1 subjective endpoints are subject to bias, especially in
2 unblinded studies.
3 (Slide)
4 Surrogate clinical endpoints are especially useful
5 in Phase II studies as markers of potential benefit. We
6 need to keep in mind that in evaluating surrogate endpoints
7 sufficient validation information is needed for the
8 relationship between the surrogate marker and acceptable
9 clinical efficacy in the therapeutic setting.
10 (Slide)
11 I will now survey of a variety of possible choices
12 of trial design and touch on just some of the advantages and
13 disadvantages in applying these designs to von Willebrand
14 disease studies. As was mentioned, design issues will be
15 considered in greater detail, we hope, during the panel
16 discussion later today.
17 Use of a parallel comparison group and blinding
18 are measures which can help reduce bias. So, we can ask the
19 question is the question is a concurrent randomized control
20 group feasible and ethical? Well, given that off-label use
21 of some products has become standard of care, use of a
22 placebo seems problematic due to the nature of the disease.
23 (Slide)
24 One ore more randomized comparison treatment

1 groups, however, may be considered if different suitable
2 products are available for study. The efficacy of the
3 standard comparison product should be well established and
4 preferably FDA approved for the indication sought.
5 Cryoprecipitate is probably not the best option due to viral
6 disease transmission risk.
7 So, when we lack and FDA approved standard for
8 comparison, one thing that we can do is to randomize
9 patients into different dosage regimen groups. We call this
10 a dose-controlled study. The doses have to be sufficiently
11 far apart, however, to show a difference between the dosage
12 groups in clinical efficacy.
13 (Slide)
14 What about concurrent non-randomized control
15 groups? Are they feasible and useful? Here we can study
16 concurrently normal patients, lacking bleeding disorders,
17 undergoing matched surgical procedures and compare bleeding
18 characteristics. Some of the problems we might encounter
19 with this type of control include: age-matched patients may
20 be available for some types of surgery but not others; the
21 recruitment of matched normals may be difficult if longer
22 than typical hospital stay is required for direct
23 observation of delayed bleeding, which is seen in this
24 clinical entity; and we have to keep in mind that the use of

1 normal controls does not establish whether patients did
2 better than they would have had treatment been withheld.
3 Normal controls do allow us to determine whether treated vWD
4 patients become indistinguishable from normals in bleeding
5 tendency under the particular dosage regimen that we are
6 studying.
7 (Slide)
8 What about historical controls? These can be, for
9 example, of at least two types. We could consider using
10 patients as their own controls if we had adequate data on
11 bleeding prior to the use of any replacement products, and
12 it was well documented, or we could compare patients to
13 historical data on normals. This might translate, in
14 practical terms, into data on patients unselected with
15 respected to bleeding disorder, for example in a surgical
16 setting.
17 This approach depends on the availability of high
18 quality historical data. As always, we need to take inter-
19 center variability into account, and we feel in any
20 discussion of using historical data it is imperative to
21 define the historical database prospectively, such that the
22 historical database must be relevant to today's practices.
23 If ancillary therapy that could have an effect on hemostasis
24 was not used during the time period of collection of the

1 historical data but is used today, then that would confound
2 our interpretation of the efficacy of the product as it is
3 being dosed. And, the historical database must be unbiased,
4 which can be hard to ensure in practice. Comparisons to
5 historical data could either be descriptive or quantitative
6 and statistical. The latter would require adequate power
7 and the pertinence of that can be discussed.
8 (Slide)
9 Let's now review again the dosage information that
10 we would like to see available for product labeling
11 purposes: The recommended usual starting dose; the usual
12 dosage range; the doses according to disease subtypes,
13 severity and indication or clinical setting; the recommended
14 dosing interval; the optimal method of adjusting dosage; and
15 the usual duration of treatment. Right now these are
16 largely empirically based and often based on replacement of
17 Factor VIII levels which, as we have seen, are not
18 necessarily always a satisfactory endpoint, particularly in
19 some settings with products that are devoid of Factor VIII.
20 (Slide)
21 In summary, some of the problems to be tackled in
22 the development of these concentrates are settling on the
23 choice of in vitro assay, and we need to determine its
24 correlation with clinical effectiveness. The assay that we

1 choose should be reliable for use in assuring lot-to-lot
2 consistency and potency. We need to determine the
3 appropriate study design to be able to correlate
4 pharmacokinetics with pharmacodynamics, that is, clinical
5 hemostatic effectiveness.
6 (Slide)
7 We also need to determine the appropriate design
8 of clinical studies to confirm and establish efficacy and
9 safety, including subject selection criteria, clinical
10 settings, surgery, spontaneous bleeding and/or prophylaxis.
11 We need to settle on the choice of an appropriate control
12 group or groups. We have to consider which study endpoints
13 are going to be most informative. We need to settle on the
14 analytical plans up front. Lastly, but certainly not least,
15 the studies need to be designed in order to provide dosing
16 information that is useful to physicians and patients.
17 Thank you.
18 DR. WEINSTEIN: The next speaker will be Dr.
19 Trevor Barrowcliffe, who is the head of the Division of
20 Hematology for the National Institute of Biological
21 Standards and Control in the U.K. Trevor's talk will be the
22 European Regulatory Perspective.
23 **European Regulatory Perspective**
24 DR. BARROWCLIFFE: Thank you very much, Mark.

1 Ladies and gentlemen, when Mark originally invited me to do
2 this talk a little while ago I told him that I was really
3 the last person to talk about this topic. So here I am,
4 more or less the last person because a few other people
5 couldn't make it.
6 The first thing to say is that there is no single
7 European perspective on licensing in relation to
8 concentrates for von Willebrand disease. The reason for
9 this is the complexities of Europe's licensing system, which
10 I have tried to set out in, hopefully, relatively simple
11 form on the first overhead.
12 (Slide)
13 We have the European Medicines Evaluation Agency,
14 the EMEA, as of about three or four years ago. They have
15 set up a centralized procedure for licensing where there is
16 one European marketing authorization, but this is only used,
17 or at least it is only obligatory for new biotechnology
18 products in the area of biologicals. The other procedures
19 that really are still in existence are the mutual
20 recognition procedure where a product is licensed in one
21 member state and then a procedure is available for
22 transferring this license, although it is not automatic, to
23 other member states. But as far as blood products are
24 concerned, we are really still talking about national

1 licensing for nearly all blood products. Certainly, that is
2 the situation as far as, for instance, using Factor VIII
3 concentrates which are already licensed for treatment for
4 von Willebrand disease.
5 (Slide)
6 To try and find out what the position is, you
7 really have to go to all of the national authorities in 15
8 member states. This is essentially what I did. I have
9 information back from most of them but not all of them.
10 So I simply asked some questions, namely, which
11 products are licensed in the various member states and, if
12 there are products which are licensed, what was the basis
13 for licensing and, thirdly, is there a declaration of the
14 von Willebrand factor content on either the package insert
15 or the label?
16 So, we are now going to do a sort of Cook's tour
17 of Europe and, if you will bear with me, we will go through
18 the various member states.
19 (Slide)
20 So, if we start with the United Kingdom, in other
21 words, the alphabetical reverse order, we have essentially
22 one product currently licensed which is 8Y. That is the
23 Factor VIII concentrate from Bio Products Laboratory, and
24 that has been licensed since 1991 for treatment of von

1 Willebrand disease. The Centeon product Haemate P was
2 originally licensed then it was taken off in 1992, not
3 because of any clinical problems or anything like that,
4 simply to do with the European regulatory requirements being
5 a bit more stringent, and I think there is an application
6 still outstanding for that. The basis for licensing was
7 essentially review of the clinical data available at that
8 time. Very recently, for the 8Y product the von Willebrand
9 factor content is now going to be declared on the label as
10 the von Willebrand factor antigen. Until now, in fact, it
11 has only been available as the dosage in relation to 8C.
12 (Slide)
13 Across the Channel to Belgium, the products
14 licensed in Belgium are the Centeon product Haemate P and
15 also the von Willebrand factor concentrate from LFB, in
16 France, which is made from Belgian plasma. The basis for
17 licensing is was review of the clinical data and there is a
18 declaration of the von Willebrand factor concentrates on the
19 label. I am not quite sure whether that refers to both
20 products or only the LFB product.
21 (Slide)
22 In The Netherlands, just one product licensed and,
23 again, it is Centeon product Haemate P. The basis for
24 licensing was a review of the clinical data and there is

1 declaration of the von Willebrand factor content in the
2 package insert.
3 (Slide)
4 I think Germany is next. There are three products
5 licensed, the Haemate HS, as it is called in Germany. It is
6 the same product, Haemate P from Centeon; also the Immunate
7 product from Immuno, and a locally produced Factor VIII
8 concentrate, NDS, produced by the German Red Cross. The
9 basis for licensing was a review of the clinical data and
10 the von Willebrand factor content of the products, and the
11 von Willebrand factor content is declared in the package
12 insert as ristocetin cofactor for Haemate HS only, not for
13 the other two products.
14 (Slide)
15 I think Italy is next. Here there are four
16 products licensed and, again, are Haemate P from Centeon,
17 locally produced Factor VIII concentrate called Emoclot, the
18 Immuno product Immunate, and quite recently I think the
19 Alpha product Alphanate. The basis for licensing was a
20 review of the clinical data and, in the case of Alphanate
21 some recent clinical trial data. The von Willebrand factor
22 content is declared in the package insert. Again, I am not
23 quite sure whether that refers to all products. It
24 certainly is for Haemate P and I think also for Alphanate.

1 (Slide)

2 Moving on to Spain, there is just one product
3 licensed. Again, it is the Centeon Haemate P. The basis
4 for licensing was stated as prospective clinical trials, and
5 the von Willebrand factor content was declared in the
6 package insert and there is a variation there to allow that
7 to be put onto the label.

8 (Slide)

9 From Spain we move north to Denmark. Here, there
10 is the one product licensed. Again, it is the Centeon
11 Haemate P. I don't have information on what the basis for
12 licensing was. It is said that the von Willebrand factor
13 content is not indicated, although we know from the other
14 countries where Haemate P licensed it is declared in the
15 package insert.

16 (Slide)

17 Now to move back down to Portugal, are no products
18 licensed in Portugal and if clinicians want to use von
19 Willebrand factor concentrate for treatment of von
20 Willebrand disease, they have special dispensation, or they
21 have to apply for dispensation for use of the product from
22 LFB, in France.

23 (Slide)

24 This is also the situation in Austria, which is

1 the next country. Again, there are no products licensed.
2 Here is the Haemate P product and also Immunate which is
3 used by special dispensation.
4 (Slide)
5 Then in Greece, again, there are no products
6 licensed and this time it is the LFB product, from France,
7 which is used by special commission.
8 (Slide)
9 Finally, two countries where I couldn't get
10 information from the national authorities but have got some
11 information from the manufacturers, in France, the LFB
12 product, the von Willebrand factor concentrate, is licensed.
13 I understand also from Dr. Mazurier that there is a second
14 product which contains also additional Factor VIII which is
15 also licensed for treatment of von Willebrand disease in
16 France.
17 In Sweden, as far as I can gather, it is just the
18 Haemate P product from Centeon, which is licensed.
19 (Slide)
20 So you can see, it is really quite a variable
21 situation in Europe with regard to licensing. So, I have
22 just summarized it here in relation to the products
23 altogether, but there are certain products which are
24 licensed in the various European countries but three of

1 these are really only available in the country of
2 manufacture. Of the first four which are potentially used
3 in more than one country, Haemate P is the one which is
4 licensed in most, but not all, European countries; then the
5 von Willebrand factor concentrate from France, which is
6 licensed in France and Belgium and is also used in two other
7 countries; the Immuno product, which is licensed in Germany
8 and Italy; and the Alpha product, which is licensed in
9 Italy; and then the three, as I said, which are only
10 licensed in the country of their manufacture, which is 8Y
11 licensed in the U.K. and Emoclot, licensed in Italy, and the
12 German Red Cross product, NDS, which is licensed in Germany.
13 So, that is the situation as far as I have been
14 able to ascertain. Some of this information was fairly
15 recently acquired, and if anybody really wants to be sure
16 about the situation in any one country I think really you
17 need to check with the licensing authority in that country.
18 Thank you.
19 DR. WEINSTEIN: Our next speaker will be Beth
20 McDonald, who is a consumer of von Willebrand-containing
21 products and has type 3 von Willebrand disease. She is
22 active in the bleeding disorder community and advocates
23 recognition of women with bleeding disorders, and is
24 involved in peer review education to ensure the quality of

1 care in this community. Beth?

2 **vWF Concentrates from Consumer Viewpoint**

3 MS. MCDONALD: Good morning. Thank you for
4 inviting me here. I am glad I get to speak but I kind of
5 wish I could have spoken at a later time because after
6 listening to all the lab values and studies that need to be
7 done to approve this product, I think it would be nice to
8 put a face to all the mumbo-jumbo that is hard for me to
9 listen to, and I want to give a personal point of view.
10 First, let me introduce myself. Beth McDonald. I
11 have type 3 von Willebrand disease, but I am also a surgical
12 nurse with Columbia Hospital, in Lexington, Kentucky. So,
13 von Willebrand's is not who I am, but it is an important
14 part of my life. I am a very productive member of my
15 community, including the surgical nurse community.
16 I want to tell you a little bit about my family.
17 I had a sister. She was a type 3 von Willebrand's and she
18 was involved in a motor vehicle accident in 1986 and then
19 they used cryoprecipitate to treat her bleeding. She
20 sustained a closed-head injury, and through the cryo the
21 hematologist that was treating her would treat her bleeding
22 time, and every time her bleeding time would be prolonged,
23 which it always was with the cryo, he would treat her with
24 more cryo and this, in turn, raised her intracranial

1 pressure. She remained comatose for ten years and finally
2 we ceased giving her blood products. By then we had gone on
3 to give her factor concentrates, and she passed away ten
4 years later through bleeding.
5 I also have two daughters, and they are six and
6 nine and they are wonderful, and they have type 1. From
7 what I understand, 1% of the population supposedly has some
8 type of von Willebrand disease, type 1 probably.
9 Through this information I can put together that
10 if you have more type 1's you are going to have more type
11 3's. If these are undiagnosed we are going to have an
12 increased incidence in type 3's. There is going to be a
13 need for these factor concentrates. We surely don't want to
14 revert back to using cryo. Cryo is time consuming. It
15 involves going to the emergency room. If I have a bleed, I
16 like to be able to infuse within 15 minutes and be back to
17 work, on my feet, and be productive. I do not want to take
18 the time out to go to the emergency room, wait for somebody
19 to order the cryo, wait for somebody to check and see that I
20 have a bleed and then get my cryo in and go home four hours,
21 six hours, sometimes 12 hours later.
22 There is also another problem with cryo. Nursing
23 homes that I have encountered, my sister having been in a
24 nursing home, they cannot hang cryo in a nursing home. So

1 we would have to go to the emergency room, and this involved
2 getting an ambulance and taking my sister out of the nursing
3 home and her environment, on a respirator. It was an
4 ordeal. When we started using the factor concentrates the
5 nurses could infuse her and this was just much easier on her
6 and on our family.

7 The big factor in all of this though is the
8 insurance issue. I have insurance coverage, luckily, and I
9 want to keep that insurance coverage. I do not want to rely
10 on my state to pay for my coverage, but the insurance
11 companies are starting to deny coverage on factor products
12 for us because it is not indicated. My treatments cost
13 $3000 per treatment. Say I make $36,000 a year, which is
14 within my realm, that would be one treatment a month. If I
15 had an injury, like a knee bleed that has put me out, that
16 would be a treatment over five days. That is going to put
17 me to receiving only seven more treatments throughout the
18 year. In other words, it will bankrupt me and my family,
19 and my insurance company will not cover that.

20 I have many friends in the hemophilia and bleeding
21 disorder community. We held a "Women with a Bleeding
22 Disorder" conference and many of us got together and we have
23 remained friends. So, I have met five other women that have
24 type 3 von Willebrand's and they remain close friends, which

1 is very nice to have.

2 The interesting thing between all of us is that we
3 treat differently. I have also met others that weren't type
4 3's and they seem to treat even more than I do. I have a
5 hard time putting a lab value on treating. I think you
6 should treat symptoms. I mean, putting a lab value --
7 severity is a description and a lab value is very
8 definitive. Severity should be how often you bleed, not
9 that if I am a severe I bleed this much. I don't. I don't
10 treat as often as my friend Marge treats, and that should be
11 an issue. It shouldn't be just black and white. I know you
12 need regulations for somebody to follow but that shouldn't
13 take priority over treating us.

14 I also want to stress that cryo is going back to
15 the Dark Ages. It is inconvenient but it also is a risk.
16 It increases our risk for hepatitis and HIV. I also have
17 experience with both cry and factor concentrates. I was
18 diagnosed at four years old. I have been through fresh-
19 frozen plasma. I have been through cryo. I now use factor
20 concentrates. I have had three procedures in my life that
21 have been major procedures, two deliveries, one vaginal, one
22 C-section, for one of those we used cryo and for one of
23 those we used factor concentrates. The cryo resulted in a
24 pleural effusion from fluid overload, just too much fluid in

1 my system, and factor was used for the C-section and my
2 bleeding was controlled. There was no regulation but it was
3 controlled and it went well. I have also had a hysterectomy
4 and we used factor, and it worked well and there was no
5 pleural effusion, no fluid overload.
6 I cannot stress enough that time is a major factor
7 here. My health care company calls me and is informing me
8 that insurance are already, today, not covering for the
9 factor concentrates. This, I have to say, would be the
10 biggest issue. Yes, I would like to see the concentrates
11 because it is the right thing to do, it is healthier and it
12 would just be more effective but I hate to say that money
13 plays a big part in all this. I do not want to have to go
14 to Medicaid to be covered, and then it would be cryo and
15 that would be even worse. I want to keep my insurance. I
16 want to be productive. I want to continue my job as a
17 surgical nurse. If you have to go back to cryo, if the
18 concentrates aren't soon enough that we will have to go back
19 to this, people will not treat as often. I think it will
20 cost the economy more because we are going to have more
21 severe injuries; we are going to be sicker. I think if we
22 keep our society healthy we need this now. We needed it a
23 long time ago. So, I hope that this will be time producing,
24 that you will take as little time as possible.

1 That is it. Thank you for having me here.
2 DR. RICK: I am Margaret Rick, part of the
3 Critical Pathology Hematology Service here, at the Clinical
4 Center at NIH. We certainly have heard an excellent
5 clinical perspective from the patient's point of view and we
6 will now turn to three different talks evaluating the
7 clinical perspective from the treaters' point of view. We
8 will have three speakers, and since I think the content is
9 relatively similar in their talks it perhaps would be best
10 to hold questions until the end of the three talks, at which
11 time we have about half an hour for a question and answer
12 period.
13 Our first speaker is Dr. Augusto Federici, who is
14 Associate Professor and a Senior Investigator in the
15 Hemophilia Center at the University of Milan. He will speak
16 to us on optimizing therapy with von Willebrand factor
17 concentrates in von Willebrand disease.
18 **Clinicians Perspective, Practice and Expectations**
19 **Optimizing Therapy with von Willebrand factor Concentrates**
20 **in von Willebrand Disease**
21 DR. FEDERICI: Thank you, Dr. Rick. It is a great
22 pleasure to be here to present our perspective and, of
23 course, I am glad and thankful to Mark Weinstein for having
24 organized such an interesting meeting.

1 I will just start with some comments on the
2 previous presentation. I think this is the issue. We are
3 dealing with patients and we have heard how difficult it is
4 for these patients to cope with their bleeding problems,
5 especially when you don't have appropriate products. So, I
6 will start with general considerations.
7 (Slide)
8 For those who are dealing with vWD these are sort
9 of dogmas: The determinant of bleeding of vWD, the prolonged
10 bleeding time is the main determinant of mucosal
11 hemorrhages. Low Factor VIII is the main determinant of
12 soft tissues and postoperative hemorrhages.
13 (Slide)
14 So simplifying everything, the aims of the
15 treatment should be to normalize the prolonged bleeding time
16 and to normalize the low plasma Factor VIII.
17 (Slide)
18 Which are our mainstays in the treatments? We
19 know, for sure, that the treatment of choice for vWD is
20 desmopressin because, as I will try to show you in the next
21 slides, most of the patients can respond very well to DDAVP.
22 However, when you don't have such a good response you have
23 to go to plasma products and in the rare situation where, in
24 the case of mucosal bleeding, you don't have such a good

1 response after plasma products there are indications in the
2 literature that platelet concentrate, very rarely, can be
3 useful.
4 (Slide)
5 Let's go to the desmopressin effect. Everybody
6 knows that it is safe, inexpensive and effective in about
7 80% of the patients. But there are limits. It is not
8 effective in about 20% of the patients, especially type 3,
9 type 1, platelet low and platelet discordant, and some cases
10 of type 2, and it can have tachyphylaxis.
11 (Slide)
12 This is a very simple history of the plasma
13 products so everybody knows how we started with the plasma,
14 the cryoprecipitate, and we are into the Factor VIII
15 concentrate.
16 (Slide)
17 This is the issue we are talking about today. So,
18 we know for sure that the virally inactivated concentrates
19 are safer than cryoprecipitate. They consistently correct
20 the Factor VIII defect and contain a large amount of vWF,
21 except for monoclonally purified concentrate, of course.
22 (Slide)
23 So, what we wanted to have in the last year was a
24 sort of impact on our patients -- how many patients treated

1 for vWD in Italy really needed Factor VIII concentrate, or
2 were happy with the desmopressin? So we applied for a grant
3 to the Instituto Superiore di Sanita, our national institute
4 of health, of course, and we received the money to organize
5 computerized retrospective information sent to the
6 hemophilia center, the treaters of vWD patients, to see how
7 many patients were actually treated with blood components
8 generally speaking.
9 This is the second evaluation. The first
10 evaluation was presented in Florence at the STH meeting.
11 There were about 1023 patients. This is a retrospective
12 analysis based on the diagnosis and the treatment performed
13 in each hemophilia center, of course. As you can see, we
14 have 65.8% of type 1, 21% type 2, 5.9% type 3, and the point
15 that our friend raised before my presentation, the point of
16 heterozygotes. These heterozygotes can be the kids of type
17 3, but they can also be misdiagnosed sometimes. If they
18 match two of these can generate type 3 vWD.
19 (Slide)
20 These are the data. I have much data, but what
21 was striking is the fact that we found that in the last 24
22 months, in the last 2 years, 29.4% of these patients,
23 divided into type 1, type 2, type 3, and heterozygotes,
24 received at least once 1 blood component and von Willebrand

1 factor concentrate altogether. When we analyzed which was
2 the most representative concentrate, it was Haemate P
3 because, as you saw before, Haemate P was already registered
4 in Italy. So, it is not surprising that between 80% and 90%
5 of type 2 were treated with these factors, but also type 2
6 and some type 1.
7 So this was an observation and so what we are now
8 analyzing is the fact that maybe we are biased by the fact
9 that the center is the treater of the most severe cases. So
10 we can have an overexposure to Factor VIII concentrate. But
11 this is one issue we have to face, the DDAVP works but
12 sometimes physicians use Factor VIII concentrates also in
13 patients with type 1 and type 2.
14 (Slide)
15 Now we are going to focus on the second point of
16 my talk. Which is the concentrate we want to deal with?
17 Going through the literature, I found that the most
18 extensive study about in vitro and pharmacokinetic analysis
19 of von Willebrand factor concentrate is still the paper by
20 Manucci, in 1992, in Blood, where he published a comparison
21 of four virus-inactivated plasma concentrates for treating
22 severe von Willebrand disease in a crossover randomized
23 trial.
24 To answer the question of the previous speakers, I

1 think that we also start with these kinds of observations,
2 and also with a paper published in Thrombosis Haemostasis,
3 in the same year where the recommendation for these analyses
4 are synthesized.
5 (Slide)
6 In that paper, the four concentrates analyzed were
7 analyzed, Factor VIII/von Willebrand factor concentrate had
8 an intact multimeric structure similar to that of normal
9 plasma or cryoprecipitate. We want to have the protein for
10 our patients that is similar to what we have in our plasma.
11 So this is a very important issue.
12 As you see, if you have a comparison to normal
13 plasma, the Alphanate at that time, Haemate P, 8Y, high
14 purity von Willebrand factor, the French concentrate. If
15 you use a correct analysis for the multimers, low resolution
16 gels that can solve very well the high molecular weight, all
17 of them show a lot of the high molecular weight multimers.
18 When you want to examine this, make sure that you have the
19 right concentration agarose. I have been working for many,
20 many years with the multimeric part and I know how to do
21 small tricks. So, if you want to resolve high molecular
22 weight multimers you have to use low resolution agarose gel.
23 So, you don't have to see the banding very much, otherwise
24 you don't solve this portion. You can have this concentrate

1 pretty much the same as normal plasma.

2 (Slide)

3 The summary of that pharmacokinetic study was that
4 all concentrates were equally effective in obtaining normal
5 and sustained levels of Factor VIII post-infusion, although
6 peak levels were more delayed in the concentrate devoid of
7 Factor VIII. We know that. But no concentrate normalized
8 the bleeding time in a sustained fashion. So this is not an
9 important issue. Bleeding time is not ristocetin cofactor.

10 (Slide)

11 As you can see, I wanted to put everything on one
12 slide but the message is very clear. All these concentrates
13 are able to correct in all the situations the ristocetin
14 cofactor activity, but the bleeding time is not corrected in
15 all the patients. So we know this and, for those who don't
16 remember, each concentrate was infused in the same patients
17 at different times. So, we know exactly what the crossover
18 is in the open trial.

19 (Slide)

20 Now I want to turn to more practical information.
21 I have been working with on von Willebrand disease for about
22 twenty years or so. I know how many patients we have and
23 how they can end their problems. So, clinical hemostasis
24 can be achieved in all types of vWD regardless of whether

1 the bleeding time is corrected. In repeated infusions, this
2 sometimes becomes a problem. Daily dosages are usually
3 decided according to Factor VIII C regardless of von
4 Willebrand factor activity.
5 So, one important issue is what we want to do when
6 we have patients in surgery. What are the levels of Factor
7 VIII C, ristocetin cofactor, and what is the amount of von
8 Willebrand factor concentrate that we want to give them?
9 (Slide)
10 I want to start with general comments about why we
11 believe that in the postsurgical situation Factor VIII C is
12 the main determinant. This is an example. It is a
13 paradoxical example. This is a patient with a severe type 3
14 vWD with alloantibody, high titer of alloantibody. She
15 responds, when you give just a shot of the von Willebrand
16 factor she has a high level of alloantibody that produces
17 immunoprecipitating immunocomplexes. This patient had a
18 very severe abdominal hemorrhage and we went to surgery.
19 These are the antibody levels. Factor VIII C is the red
20 line. The blue line is the ristocetin cofactor activity.
21 We used the most potent concentrate available, namely, the
22 Haemate P, and she went to surgery and she could go through
23 the surgery. So, we had von Willebrand factor and Factor
24 VIII C at the same time.

1 Of course, she had a sort of anaphylactic
2 reaction. We could manage that with the help of the people
3 in the emergency area. You see, you have a very good
4 response, but because of the alloantibody the response to
5 von Willebrand factor was pretty poor but, still, we had
6 some response with Factor VIII C. We went to day 7. But
7 when we had such a rise in the antibody we were not able to
8 give any more von Willebrand factor.
9 (Slide)
10 Most of the people know this slide. The only
11 chance to have a solution for this patient was to turn to
12 recombinant Factor VIII devoid of von Willebrand factor this
13 time. You see, since we know for sure that if you don't
14 have von Willebrand factor around you have a very short
15 half-life, we had to turn to continuous infusion of Factor
16 VIII C, and we managed that.
17 So, if you give Factor VIII C and you keep the
18 amount of Factor VIII C at about 50 U/dl you can manage.
19 You can go out of surgery.
20 (Slide)
21 But this comes with problems. When we are dealing
22 with Factor VIII/von Willebrand factor concentrate one of
23 the important thing is to remember that if you give a
24 concentrate containing both von Willebrand factor and Factor

1 VIII C to a patient with vWD you have such a delay response
2 to Factor VIII C.
3 So, when we are going to treat patients with
4 severe von Willebrand disease type 2 or type 1, we have to
5 consider that. This is a case report published in Vox
6 Sanguina, by Eric Bertorp. During the operation he was
7 giving Haemate P, or whatever. I am talking about Haemate P
8 but it would be the same with other concentrates containing
9 Factor VIII C and von Willebrand factor. You really have to
10 deal with the fact that if you give many infusions -- at
11 this time he had a good response in bleeding measured by
12 Duke bleeding time, and a lot of von Willebrand factor
13 around, and more than 500 units in the postsurgical time.
14 So, this is another issue we have to deal with.
15 (Slide)
16 This is another case report on efficacy studying,
17 you know, several infusions of a concentrate containing both
18 Factor VIII C and von Willebrand factor. It reminds you of
19 one thing, that the first thing we usually do is give for
20 the first two or three days a daily infusion in order to
21 have enough Factor VIII C and von Willebrand factor around.
22 But when we see that Factor VIII C and ristocetin cofactor
23 activity is very high, we usually go to every other day.
24 Only if there is a bleeding problem we try to go back to the

1 daily infusion.

2 That means that when you have to deal with
3 patients, especially in emergency, you cannot make von
4 Willebrand factor antigen. We should have a sort of very
5 quick assay, and it can be Factor VIII C because actually in
6 the postop. situation Factor VIII C is the main determinant.
7 So, practically, we don't do ristocetin cofactor and von
8 Willebrand factor antigen immediately. We do it for other
9 purposes, for pharmacokinetics, but we use Factor VIII C as
10 the marker of our next infusion.

11 (Slide)

12 This is another example. This is a patient with
13 type 2A, a very old patient. She has 2A with very prolonged
14 bleeding time, more than 30 minutes, ristocetin cofactor
15 lower than the technical limits, von Willebrand factor
16 antigen about 50 and Factor VIII C about 70%. She had a
17 heart attack. She went through surgery. She had coronary-
18 artery bypass surgery.

19 As you can see, she was 71 years old. DDAVP was
20 impossible. So, in this case we used Haemate P and we
21 adjusted the dose based on the Factor VIII C levels. You
22 see that on the first day we gave Haemate P twice in order
23 to have such an increase in Factor VIII C. Then we went to
24 this kind of shorter dosaging in order to keep the amount of

1 Factor VIII C between 70-100, no more than that.

2 So, this is another situation where you have a
3 sort of good response for the patient by giving Factor VIII
4 vWD concentrate and by monitoring just by Factor VIII -- no
5 bleeding time at all. And you have to deal with a surgeon
6 who is very concerned about bleeding.

7 (Slide)

8 But there is another issue, the gastrointestinal
9 bleeding. I don't know if the audience has experience with
10 vWD and gastrointestinal bleeding. They come to you many
11 times. They stay in the hospital for a very long time and
12 they require sometimes months of treatment.

13 We have had a few patients with this situation and
14 most of them were treated very well with Factor VIII
15 concentrates. But, in a situation where you don't cope with
16 bleeding, there is one extra emergency treatment, the
17 platelet concentrate, as published by Castillo in 1991. I
18 recall for those of you who don't remember, the fact that
19 the experiment by Castillo was pretty nice. So, he treated
20 with cryoprecipitate first and he had a very nice correction
21 of ristocetin cofactor but still, in some patients, the
22 bleeding time was prolonged. So, after that he gave these
23 patients platelet concentrate and, as you can see, the
24 bleeding time was fully corrected in spite of the reduction

1 of ristocetin cofactor since the platelet concentrate did
2 not provide enough von Willebrand factor.
3 (Slide)
4 These are the conclusions and the expectations at
5 the same time. How to avoid the loss of high molecular
6 weight multimers in the preparation of our concentrate? You
7 know, all the doctors who are going to treat patients with
8 vWD, and also the patients, would like to have an intact von
9 Willebrand factor.
10 One other thing we should evaluate is the Factor
11 VIII C pharmacokinetics following concentrate in vWD, and in
12 this issue indication for pure von Willebrand factor devoid
13 of Factor VIII C, if there are indications.
14 (Slide)
15 I want to conclude by saying to you that because
16 we are concerned about these problems, Prof. Manucci and I,
17 together with other groups in France, London, Frankfurt,
18 have applied to the European Community to have a study
19 called "Optimized Orphan Drug Therapy in Severe Forms of
20 vWD," because we want to see if those patients who are
21 unresponsive with vWD would have the best treatment.
22 (Slide)
23 These are the goals: to evaluate the proportion of
24 the vWD unresponsive patients who require Factor VIII/von

1 Willebrand factor concentrate; to test the new plasma-
2 derived von Willebrand factor concentrate devoid of Factor
3 VIII in a crossover pharmacokinetic study with the Factor
4 VIII concentrate used in different European countries.
5 So, I will thank you for your attention with this
6 kind of message. Thank you.
7 DR. RICK: Thank you. We will now move along to
8 the first of our two survey reports for clinical guidelines.
9 Our first speaker will be Dr. Jeanne Lusher, who is the
10 Chairman of the National Hemophilia Foundation Medical and
11 Scientific Advisory Council, as well as a Marion Bernhardt
12 Research Professor at Wayne State University. Dr. Lusher?

13 **Clinical Guidelines for Treating von Willebrand Disease**
14 **Patients Who are Not Candidates for DDAVP**
15 **Survey Responses from European Physicians**

16 DR. LUSHER: Thanks very much, Dr. Rick. I think
17 this is a fascinating conference, with a very impressive
18 agenda and, hopefully, we will all leave here with some
19 better understanding.
20 In terms of these surveys, just a bit of the
21 background of the surveys, in the United States we began
22 hearing rumors or reports of patients with severe von
23 Willebrand disease being denied payment for their clotting
24 factor concentrate because it wasn't a licensed indication.

1 I don't think this has become widespread yet but at least it
2 is occurring in certain parts of our country.
3 Perhaps as a result of this or in anticipation of
4 this, at least two companies, at least two manufacturers,
5 have either applied to the FDA, or are planning to, or are
6 in the process of applying to the FDA for licensed
7 indications for von Willebrand disease for their products.
8 So, when the Medical and Scientific Advisory
9 Council of the National Hemophilia Foundation had its last
10 meeting, this past spring, Dr. Mark Weinstein, who is a
11 member of our group, asked that we survey what various
12 esteemed treaters were doing in this regard since there did
13 not seem to be really clear-cut studies indicating what
14 dosage should be used, which products were best, and there
15 were certainly apparent problems with some of the assay
16 methodologies, particularly for von Willebrand factor.
17 So, in response to Mark's request, Dr. Kessler, as
18 you will hear in the next presentation being given by Dr.
19 Cohen, Dr. Kessler embarked on a survey questionnaire which
20 has been used in the United States and, in talking to Mark,
21 I thought it would be interesting to look at the experiences
22 in Europe since many of these products, particularly Humate-
23 P, have been used for many, many years by European treaters
24 and they have written a lot of articles on proper dosages,

1 and how to monitor patients. So, I then surveyed what I
2 viewed as a very experienced, esteemed group of European
3 colleagues who have written on von Willebrand factor disease
4 or are very active in the field to see what exactly they
5 were doing.
6 (Slide)
7 This is a survey of clinical guidelines for
8 treating von Willebrand disease patients who were
9 unresponsive to DDAVP. I sent this questionnaire to 30
10 European physicians. I now have responses back from 27 but
11 the last few just came in, in the past week and I didn't get
12 them on the slides but, in looking at them, they really do
13 not change the message of these responses.
14 At the time that I made the slides up I had
15 received responses from nine countries. I have subsequently
16 received responses from two additional European countries
17 not listed here. But the ones in the slides are from
18 Austria, Denmark, France, Germany, Italy, The Netherlands,
19 Sweden, U.K. and, not European, but I decided as an
20 afterthought to send this to some Japanese treaters as well
21 as Australians and I have just recently received their
22 responses.
23 (Slide)
24 One of the first questions asked was which

1 concentrates do you feel are useful in treating persons with
2 von Willebrand who are unresponsive to DDAVP? As you can
3 see, the majority of these European respondents said that
4 they felt that either Humate-P or the French von Willebrand
5 concentrate were most effective. One of the U.K. persons
6 said 8Y. Interestingly, other U.K. persons didn't list 8Y.
7 Actually, there are two now who indicated Alphanate SD. I
8 think that reflects, in large part, what has been available
9 in various European countries and, certainly, Alphanate, as
10 we saw earlier this morning, is not very widely available in
11 Europe.
12 (Slide)
13 In fact, if we look at those who checked off the
14 French von Willebrand factor concentrate as being very
15 useful, seven persons from France -- in fact, I got my
16 greatest response from the French physicians and I must say
17 that without exception the French physicians are extremely
18 enthusiastic about their French von Willebrand factor
19 concentrate, several of them writing patients describing how
20 wonderful it was and that we should get used to it here and,
21 hopefully, that some day we would have it available in the
22 U.S. So, they feel that that works extremely well. Also,
23 we got responses from Denmark and U.K. saying that they felt
24 that this worked very well, not necessarily from personal

1 experience but from looking at data.

2 (Slide)

3 I also asked do you ever use cryoprecipitates,

4 like in the 1990s? Are you using cryoprecipitates for such

5 patients? All of them said emphatically no, and many wrote

6 in that they felt that compared to the other concentrates,

7 which are all treated, they were really concerned about

8 viral safety. In some instances they even indicated that

9 cryoprecipitates are no longer available to them.

10 (Slide)

11 The next question was how do you decide on the

12 dosage to be used in a couple of different situations, first

13 looking at major surgical procedures? Interestingly or

14 perhaps expectedly, most did not make a distinction between

15 how they treated severe or type 3 patients and type 2 von

16 Willebrand disease patients. The type 2's were unresponsive

17 to DDAVP, ones who had very low levels of von Willebrand

18 factor.

19 Six stated that they aimed for a certain Factor

20 VIII level only, and the levels that they aimed at in the

21 responses were either greater than 50% -- some put in just

22 greater than 50% or 80%, up to 100%. But this was the range

23 in all that people would calculate based on the label, and

24 they would aim for a level in the recipient somewhere over

1 50% or 100% for surgery.

2 Three of them aimed for a certain ristocetin
3 cofactor level only, and the level they aimed for surgery
4 was 60-80%. Most, or eleven of these respondents, aim for
5 both a Factor VIII and a cofactor level, again, in the same
6 ranges. Eleven would like to see not only the Factor VIII
7 be greater than 50% up to 100%, but the von Willebrand
8 factor as measured by ristocetin cofactor.

9 (Slide)

10 What about for mucous membrane bleeding? Again,
11 most did not make a distinction between severe and type 2
12 von Willebrand disease, but several did write in that they
13 distinguish between GI bleeding and epistaxis. I hadn't
14 asked that but I think it makes sense that one would make a
15 distinction there where one can look and see if the patient
16 is still having nose bleeding, whereas the gastrointestinal
17 bleeding may be much more problematic and it is not that
18 easy to see how much is still going on in all instances.
19 So, many of them said that they would tend to treat with
20 higher levels and aim for higher levels for gastrointestinal
21 bleeding than they would for epistaxis where they could see
22 what was going on.

23 Again, some said that they would aim for a certain
24 Factor VIII level only, again in the same range of 50-100%.

1 A few people aim for a certain ristocetin cofactor level
2 only, 20-100%, the 20% being for epistaxis and the higher
3 values, it was usually written in, used for gastrointestinal
4 bleeding. Only one would also like to see correction of the
5 bleeding time. In fact, most respondents didn't do bleeding
6 times at all in the recipients. Four aim for both a certain
7 level of Factor VIII and ristocetin cofactor, one with
8 bleeding time.
9 A number of them gave an empiric dose of somewhere
10 between 20-60 U/kg and just looked for control of bleeding
11 without monitoring any particular test. Only two would do
12 bleeding times. So, many would give an empiric dose and if
13 it seemed to control the mucous membrane bleeding,
14 particularly if it was epistaxis, no tests were obtained.
15 (Slide)
16 As far as monitoring, we asked how would you
17 monitor the patient who has just undergone major surgery?
18 None here made a distinction between type 3 and type 2 von
19 Willebrand disease. They indicated that their monitoring
20 was pretty much the same. Two follow the ristocetin
21 cofactor only, doing this once daily. Three follow the
22 activator partial thromboplastin time and Factor VIII only,
23 doing this once daily. One does Factor VIII daily plus a
24 bleeding time on the first day only. Five do both Factor

1 VIII and ristocetin cofactor assays daily. One does a
2 combination of Factor VIII, von Willebrand factor antigen
3 and bleeding time. These are done once daily but the
4 bleeding time is done every 12 hours for the first day and
5 then PRN thereafter. Two did Factor VIII antigens and
6 cofactor assays daily. One did Factor VIII, cofactor and
7 bleeding time daily. One did Factor VIII, cofactor,
8 antigen, bleeding time and the collagen testing daily.
9 You can see here quite a variety of choices, and
10 many people wrote in that they really distinguished
11 individuals depending on the extent of the bleeding the
12 person was having. They monitor these on all of these
13 parameters. They may do only a few of them if the patient's
14 bleeding seems readily controlled. So, there is a lot of
15 individual variation among the respondents from what they
16 wrote in. They said that it was difficult to generalize for
17 each individual because they do look at each in terms of the
18 extent of the bleeding and how readily it was collected.
19 Several wrote in that even though they obtain this
20 whole battery of tests, they don't get the test results
21 back, in the most part, for perhaps two or three days. For
22 instance, the collagen binding assays, and even in some
23 instances the ristocetin cofactor assays. So they use these
24 for retrospective analyses and to correlate with how the

1 patient's response has been on the dose they had given, but
2 they do not really have this full range of test results back
3 in order to make daily decisions as far as dosage.
4 One who responded in this fashion, of several who
5 wrote this in, is Prof. Jenny Goudemand from whom we will
6 hear later and perhaps she can tell us how those things have
7 correlated. But in many instances the only thing that is
8 readily available in order to make the decision as far as
9 the next dose is the Factor VIII activity and, for those who
10 do it, of course the bleeding time but they are in the
11 minority. The collage binding assays, done by a few people,
12 are generally not available for making any clinical
13 decisions.
14 (Slide)
15 As far as monitoring for major surgery in general
16 then, if we can summarize what tests were used even though
17 they were used in various combinations by the various
18 respondents, Factor VIII was certainly used by the majority
19 for monitoring their patients postoperatively. We see here
20 that 18/20 followed Factor VIII as far as monitoring and
21 generally this was done daily; 3/18 follow Factor VIII only
22 with the APPT; 1 other measured Factor VIII and bleeding
23 time, the bleeding time being done on the first day only.
24 Ristocetin cofactor assays, two followed

1 ristocetin cofactor only, doing it daily. Six followed
2 Factor VIII plus ristocetin cofactor. Three followed Factor
3 VIII, von Willebrand factor, the cofactor and the antigen,
4 two of this group with bleeding times, and two with collagen
5 binding assays as well, again, not getting the results back
6 on the collagen binding assays and many times not getting
7 the cofactor or the antigen back until at least a day later.
8 Bleeding times were only used for monitoring in
9 4/20. In looking at the additional responses I received
10 after making up the slides, there were no additional people
11 who checked off that they monitor bleeding times. So, it
12 seems to be a relatively small proportion who monitor the
13 bleeding time postoperatively. Of those who do it, one did
14 it on the first day only; one day did it every 12 hours for
15 the first day and then daily; and two said they did it daily
16 until they were sure that the patient was well controlled.
17 (Slide)
18 Several respondents noted, as I have mentioned,
19 that they obtain multiple tests but the results come back
20 one to two days later, therefore, they use only the Factor
21 VIII and, in some instances, the aPTT as well and
22 occasionally a bleeding time to make clinical decisions.
23 Many times they responded more to how the patient was doing
24 in terms of any bleeding or lack thereof.

1 (Slide)

2 What about monitoring the patient who has had
3 significant mucous membrane bleeding, gastrointestinal
4 bleeding, epistaxis? Six stated that they usually look for
5 cessation of bleeding only. A seventh would do this for
6 epistaxis, in other words, look for cessation of bleeding
7 only. Six do aPTTs and a seventh would do aPTTs for GI
8 bleeding. Ten stated that they monitor hemoglobins and
9 hematocrits and that these are particularly useful in
10 gastrointestinal bleeding for monitoring response to
11 treatment. Eleven monitor both Factor VIII and ristocetin
12 cofactor. Fifteen monitor Factor VIII. Thirteen monitor
13 ristocetin cofactor and some the bleeding time, usually a
14 modified IV bleeding time, and two with collagen binding
15 assays.

16 (Slide)

17 In asking finally what information do you feel
18 useful to have on the label? Most of us, at least in the
19 U.S., have only Factor VIII on the label and, as we heard
20 from Dr. Barrowcliffe, some of the European products do have
21 ristocetin cofactor at least on the package insert, if not
22 on the label itself. But what would clinicians like to have
23 on the label in addition to Factor VIII?

24 Most said that they would like to have the

1 ristocetin cofactor, realizing the problems in assaying that
2 and, hopefully, those assay problems could be resolved. But
3 if it were a really bona fide ristocetin cofactor content in
4 the product, they would like to see that on the label.
5 Thirteen of twenty said they would like also to
6 see the range of multimers, in other words, to know whether
7 or not the highest molecular weight multimers were present.
8 Only 9/20 said that they would like to have the von
9 Willebrand factor antigen content on the label, and 2/20
10 said that they would find it very useful to have the
11 collagen binding assay information as well.
12 This is sort of an overview of the survey. I am
13 going to try to summarize this in a better form for Mark
14 Weinstein and send it to him because the letters that
15 accompanied this I thought were very useful in seeing
16 exactly how European physicians, who are very experienced,
17 really thought about each individual patient and how they
18 should be monitored.
19 Thank you, and I would also certainly like to
20 thank all of the Europeans who took the time to answer the
21 questionnaire. I thought it was extremely useful.
22 DR. RICK: Thank you. The second half of our
23 survey information will be presented by Dr. Alice Cohen, who
24 is Assistant Clinical Professor of Medicine at Columbia

1 University College of Physicians and Surgeons. Dr. Cohen?

2 **Current Practice Patterns for the Management**

3 **of von Willebrand Disease**

4 DR. COHEN: Thank you, Dr. Rick. Good morning. I

5 appreciate being invited to present the preliminary results

6 of our survey, and I would also like to thank Dr. Craig

7 Kessler and Dr. Bruce Ewenstein who helped with the

8 development of the questionnaires that we will be discussing

9 today, and for their assistance in reviewing some of this

10 preliminary data.

11 (Slide)

12 The optimum treatment of patients with von

13 Willebrand disease remains to be defined. Moreover, it has

14 not been firmly established which, if any, of the commonly

15 measured parameters of von Willebrand factor activity in the

16 blood are useful in guiding this therapy.

17 (Slide)

18 In order to better understand what guidelines

19 physicians follow for the management of von Willebrand

20 disease patients, this study was designed to evaluate

21 current practice patterns of North American physicians who

22 are frequent treaters of patients with von Willebrand

23 disease. In addition, the need for a specific von

24 Willebrand factor replacement product for treatment of von

1 Willebrand disease was assessed.

2 (Slide)

3 Two questionnaires were developed and sent to 201
4 active patients of the Hemophilia Research Society. Any of
5 you who have not returned that survey, please do. The first
6 questionnaire, consisting of 17 questions addressing
7 diagnosis and general treatment guidelines, was to be
8 completely completed within a two-week time period. The
9 second questionnaire, consisting of 37 questions, was to be
10 completed within six weeks and addressed details about the
11 treatment of specific types of von Willebrand disease.

12 The preliminary results from the first of these
13 questionnaires are being presented today and include the
14 responses of 57 returned questionnaires, a 28% response
15 rate. Since this data was looked at last week we have
16 received an additional 10-12 questionnaires, and I looked at
17 that data and it pretty much parallels the data that will be
18 presented today.

19 (Slide)

20 The areas of investigation of the first
21 questionnaire included laboratory diagnostic testing for von
22 Willebrand's disease, laboratory monitoring pre- and post-
23 treatment, the treatment of patients refractory to DDAVP,
24 the prevalence of infectious complications after infusion of

1 plasma-derived von Willebrand factor replacement products,
2 and the identification of the variables important in the
3 selection of a von Willebrand factor replacement product by
4 treaters.

5 (Slide)

6 Treaters were asked to select the laboratory tests
7 which they utilized to make the diagnosis of von Willebrand
8 disease and to indicate the frequency of use of each test in
9 their diagnostic evaluation.

10 This slide lists the percentage of treaters that
11 would always utilize an individual test. Treaters were also
12 asked if they utilized tests sometimes or never, and I am
13 presenting the data of the most frequently used tests. In
14 this slide the von Willebrand factor activity used by almost
15 all treaters was the ristocetin cofactor activity, and I
16 will refer to it as von Willebrand factor activity in all of
17 the rest of the slides. Factor VIII and von Willebrand
18 factor antigen were employed by 100% of the treaters, and
19 von Willebrand factor activity by 98% of the treaters to
20 make the diagnosis of von Willebrand disease. Seventy-five
21 percent of treaters utilized the aPTT and the platelet count
22 when making the diagnosis of von Willebrand disease.

23 Less frequently utilized tests were the bleeding
24 time, multimeric analysis, prothrombin time, blood type and

1 ristocetin platelet agglutination. As seen on this slide,
2 the bleeding time was used only 49% of the time boy our
3 treaters to make the diagnosis of von Willebrand disease.
4 (Slide)
5 The cost of performing diagnostic tests was
6 extremely variable. Excluding treaters from Canada, the
7 cost ranged from $100 to $1000 dollars, with 49% of treaters
8 reporting the cost at greater than $500. Treaters reported
9 that the cost was variable even within their own center,
10 depending on the workup selected for an individual patient,
11 and in particular, those treaters that were utilizing
12 multimeric analysis obviously had a much higher cost for
13 making their initial diagnosis.
14 (Slide)
15 Fifty-eight percent of treaters reported
16 difficulty with reimbursement for diagnostic testing,
17 however, this rarely impacted on their selection of the
18 diagnostic tests performed. Most of the difficulty related
19 to managed care companies' requirements for authorization or
20 for the use of a particular laboratory site for the testing.
21 That is, insurance companies would tell some of the treaters
22 that they could not perform the test on site and they had to
23 perform the test at an outside laboratory. Some treaters
24 reported that testing performed on site could allow them to

1 write-off their charges when insurance companies would not
2 reimburse them so they were still able to continue doing the
3 test at this time though cost was a major problem that they
4 anticipated for the future.
5 (Slide)
6 In patients previously diagnosed with von
7 Willebrand's disease repeat laboratory testing was performed
8 only prior to major surgery. Testing prior to minor
9 surgery, dental extractions and postpartum was performed
10 extremely infrequently. Prior to major surgery the tests
11 utilized by most treaters are the Factor VIII and the von
12 Willebrand factor activity and the platelet count. The
13 Factor VIII C was utilized approximately 60% of the time
14 prior to major surgery, the von Willebrand factor and
15 ristocetin cofactor activity 60%, and the platelet count
16 52%.
17 (Slide)
18 When testing was performed to monitor treatment,
19 most treaters tried to achieve levels of Factor VIII, von
20 Willebrand factor activity and von Willebrand factor antigen
21 of greater than 80% for patients undergoing major surgery or
22 trauma and for the treatment of central nervous system
23 bleeding.
24 For patients undergoing minor surgery or dental

1 extractions or patients with menorrhagia, the goal of
2 treatment for most treaters was to increase Factor VIII and
3 von Willebrand factor activity to between 50-80%. Though we
4 said most people were not measuring levels, when they were
5 asked the question what the goal of their treatment was they
6 did admit to measuring levels sometimes and they would try
7 to achieve them to this level.

8 For treatment of mucous membrane dealing the goal
9 of treatment ranged from between 20-80% and there was a very
10 wide range of levels that people would use. As Dr. Lusher
11 mentioned, many of the comments were that it depends on the
12 individual patient to what level they would treat the
13 patient.

14 For Factor VIII, von Willebrand factor activity
15 and antigen, this then ranged between 20-80%, and the
16 bleeding time was used extremely infrequently to monitor
17 patients postoperatively. Even with major surgery it was
18 used only about 25% of the time.

19 (Slide)

20 We did ask patients if they utilized DDAVP what
21 kind of laboratory testing they performed. Prior to
22 therapeutic use of DDAVP, 93% of the treaters gave a test
23 dose. In these patients that were then given intravenous
24 DDAVP, if they were responsive 67% of the treaters felt that

1 if they were to switch to intranasal use of DDAVP they would
2 retest these patients. For patients who were documented to
3 be responders to intranasal DDAVP, 19% of the treaters then
4 would retest them if they switched to intravenous use. The
5 majority of treaters found that 10% of their patients who
6 responded to intravenous DDAVP use did not respond to
7 intranasal use, and 45% of physicians felt that age played a
8 role in this non-responsiveness to intranasal DDAVP. Some
9 of the suggestions were that the child was not utilizing the
10 intranasal use appropriately.
11 (Slide)
12 For patients who are inappropriate for treatment
13 with DDAVP, 96% of treaters utilized a pasteurized or
14 solvent detergent-treated intermediate-purity Factor VIII
15 concentration. Only 3% of our treaters utilize
16 cryoprecipitate; 2% utilize a recombinant Factor VIII
17 concentrate, and this was in an allergic patient; and 2%
18 utilize an investigational von Willebrand factor
19 concentrate.
20 The intermediate-purity Factor VIII concentrates
21 that are utilized in this country included Humate-P,
22 Alphanate and co-8-HP, and they were used in different
23 percentages depending on the location and the availability
24 of the product in each hospital.

1 (Slide)

2 To calculate the dose of a Factor VIII concentrate
3 to be administered, physicians utilized one or more of the
4 following parameters: the Factor VIII was utilized 77% of
5 the time; the von Willebrand factor, ristocetin cofactor
6 activity was utilized 31% of the time; and the von
7 Willebrand factor antigen was utilized 2% of the time. And
8 75% of treaters tried to achieve a Factor VIII C level of
9 greater than 50% post-infusion of their particular Factor
10 VIII concentrate. However, 65% of treaters do not believe
11 that the Factor VIII content is an accurate representation
12 of the von Willebrand factor activity in the product.

13 Adjustment of the dose of replacement product was
14 done empirically by 15% of the treaters, and also as Dr.
15 Lusher had mentioned, we had comments written in on our
16 questionnaire that many times the test results were not
17 available for them to make a determination as to when the
18 next dose would be, or if a next dose was necessary.

19 Eighty-six percent of treaters utilized laboratory
20 tests to predict efficacy, and 88% believe that labeling of
21 the vial with von Willebrand factor activity would be
22 helpful in selecting the appropriate dose for treatment.

23 (Slide)

24 Infectious complications in von Willebrand disease

1 have been seen in all age groups, the greatest number in
2 patients aged between 12 and 50. In this age group, 79% of
3 treaters reported at least one case of hepatitis C; 35%
4 reported at least one case of HIV infection; 28% reported at
5 least one case of hepatitis B; 10% hepatitis A; and 5%
6 parvovirus infection.
7 As you can see, there were infections across all
8 age groups and on this slide you can see that there were
9 infections in children less than 12 as well as over 50.
10 (Slide)
11 This slide describes the prevalence of infections
12 and the relationship to the prior treatment. Most treaters
13 report HIV, hepatitis A, B and C, and parvovirus infection
14 in patients with von Willebrand's disease who had been
15 treated with cryoprecipitate and/or an intermediate-purity
16 Factor VIII concentrate.
17 As you can see from this slide, most of the
18 infections were occurring in patients that had been treated
19 with cryoprecipitate, but there is overlap in that some of
20 the patients that had been previously treated with
21 cryoprecipitate also received intermediate-purity Factor
22 VIII concentrates. There were infections reported from
23 other blood products as well.
24 (Slide)

1 When asked to rank variables as to the importance
2 in selection of von Willebrand factor replacement product,
3 more than 95% of responders felt that viral attenuation
4 techniques and clinical proof of lack of transmission of
5 HIV, hepatitis B and hepatitis C were very important.
6 Greater than 80% selected PCR examination of the final
7 material and clinical proof of lack of transmission of HIV
8 and parvovirus as moderately or severely important.
9 (Slide)
10 As far as other factors unrelated to infection,
11 the integrity of the von Willebrand factor multimeric
12 structure, purity and cost of replacement products were
13 rated by greater than 80% as moderately or very important.
14 (Slide)
15 Preliminary results from this study reveal that
16 most von Willebrand disease treaters utilize the Factor
17 VIII, the von Willebrand factor ristocetin cofactor
18 activity, the von Willebrand factor antigen, the aPTT and
19 the platelet count to diagnose von Willebrand disease, with
20 bleeding times, ristocetin-induced platelet aggregation and
21 multimeric analysis used infrequently.
22 The cost of laboratory testing is high and,
23 therefore, is performed only prior to major surgery for
24 previously diagnosed patients.

1 Despite the unavailability of a licensed von
2 Willebrand factor replacement product, most treaters select
3 a pasteurized or solvent-detergent treated intermediate-
4 purity Factor VIII concentrate rather than cryoprecipitate
5 because of the desire for viral safety.
6 Post-treatment laboratory monitoring is performed
7 to predict efficacy despite the lack of studies to define
8 the therapeutic dosage and duration of treatment in
9 different clinical settings.
10 The data presented today supports the need for
11 controlled trials with prospective viral surveillance to
12 define how to better utilize and monitor safe von Willebrand
13 factor replacement products. Because of the desire by
14 treaters to have safe products made available as soon as
15 possible, the compilation of experience of treaters from
16 many centers, as in this study, may provide information that
17 would assist in the development of guidelines for management
18 of patients with von Willebrand's disease, and the design of
19 future clinical trials. We look forward to the second
20 questionnaire to look for more specific details about how
21 physicians are treating patients with specific types of von
22 Willebrand disease. Thank you.
23 DR. RICK: There is now time for an open
24 discussion, and I would ask perhaps that the speakers,

1 including not only the last three but those who went before
2 also, to either sit near the front or near a microphone.
3 There are two microphones set up in the aisles. I would
4 like to just open the discussion with questions.

5 **Question and Answer Period**

6 DR. WEINSTEIN: Actually, I wanted to ask Trevor,
7 when you mentioned that von Willebrand factor content was
8 put on European labels, was that just referring to the
9 ristocetin cofactor activity, or was it antigen levels?
10 DR. BARROWCLIFFE: I think it is mostly the
11 ristocetin cofactor activity, but I don't have the full
12 information on all products in all countries, but certainly
13 in Germany the package insert does have the ristocetin
14 cofactor activity. This is for the Humate-P product. In
15 the U.K., as I said, just very recently it is the von
16 Willebrand factor antigen that is going to go on the label
17 for the 8Y product.
18 DR. WEINSTEIN: When you mentioned that they had
19 the trials there for the clinical evaluation -- I forget
20 exactly what the wording was here, studies that were
21 reviewed by the European authorities, do you have any sense
22 of how that review was done? They just looked at papers and
23 said this is okay? Do you have further explanation about
24 what was done?

1 DR. BARROWCLIFFE: I think that is the question
2 that really needs to be addressed in much more detail. I am
3 not the person involved in those decisions but, certainly, I
4 think the various licensing authorities would be reviewing
5 all of the published evidence and, presumably, any
6 unpublished clinical data. I mean, there may be other
7 people in the room who can comment, either from the
8 manufacturers or any of the other licensing authorities.
9 But I didn't get any detailed responses to that in the
10 questionnaire. So, it is really a bit difficult to say.
11 DR. LUSHER: I have a follow-up question for Dr.
12 Barrowcliffe. Since, you know, we have all heard of the
13 problems in assaying ristocetin cofactor and standards, and
14 so forth, in the products in Europe that do have a notation
15 somewhere in the package insert of the ristocetin cofactor
16 content, is that done with a certain European standard or
17 NIBSC standard, or are they just manufacturer dependent, or
18 how is that being done?
19 DR. BARROWCLIFFE: There is no European standard,
20 as far as I am aware, and, as you know, there is no
21 concentrate standard. So, as far as I know, the ones who
22 are doing the ristocetin cofactor activity in concentrates
23 use the WHO plasma standard for ristocetin cofactor as the
24 basis for the unitage, but it may be that at least some of

1 the manufacturers probably have in-house concentrate
2 reference materials. But the basis for the unitage is the
3 WHO plasma standard for ristocetin cofactor.
4 DR. RICK: Please come to the microphone to ask
5 questions, and also identify yourself, if you would.
6 DR. WHITE: White, Chapel Hill. I guess I am a
7 little surprised that the responses regarding bleeding time,
8 and would have thought that people would have checked
9 bleeding times perhaps a little more often. Obviously, it
10 is difficult to do a bleeding time but we all talk about the
11 defect in von Willebrand's disease, and it is a platelet
12 adhesion defect. So, I guess I would like to stimulate a
13 little more discussion about the bleeding time and what role
14 the bleeding time should play.
15 I wanted to ask Dr. Federici a couple of questions
16 about his presentation. The first is, he says he doesn't
17 check the bleeding time, but I wonder if he checks the
18 bleeding time with a given product in a patient at some
19 point in time, maybe years before surgery but at least at
20 some point in time to ensure that a patient does respond to
21 that product with bleeding time correction.
22 The second question that I have for him has to do
23 with the comment that I thought I heard him saying, that
24 there wasn't a good correlation between bleeding time and

1 ristocetin cofactor correction, and that it was the
2 ristocetin cofactor correction that he thought correlated
3 better with prediction of hemostasis following surgery.
4 DR. WEINSTEIN: Perhaps we could ask Dr. Federici
5 to respond before the other questions.
6 DR. FEDERICI: I think one major point we have to
7 state is the fact that there are two different situations.
8 Okay? And the information also comes from the questionnaire
9 we sent to the centers. I mean, we have to make a
10 distinction between the moment of diagnosis and the moment
11 of when you have the patients in surgery or in emergency. I
12 am not thinking that the bleeding time is not important, but
13 I also think that sometimes it is difficult, or is it is not
14 convenient in general practice to repeat the bleeding time
15 as a monitoring system.
16 So, what we usually do -- and we have been
17 following more than 300 patients, and for those patients,
18 especially type 3, type 2, those who bleed and require
19 several appointments, most of them are routinely followed in
20 checkups, and we know the basal level. We have it in the
21 computer. We know exactly what they are going to be. So, I
22 am not thinking that the bleeding times or other assays
23 cannot be repeated sometimes, but I am saying that when you
24 have a postsurgical situation, if you follow, if the surgeon

1 is a good surgeon and can correct hemostasis during the
2 surgery you don't need to repeat the day after the surgery
3 the bleeding time. It is not convenient sometimes and I
4 believe it is not so important because what you want to keep
5 is the coagulation of this patient in a good position.
6 DR. RICK: Before you leave the microphone, could
7 I just ask do you think it is, indeed, necessary to correct
8 the bleeding time, whether you have been monitoring it or
9 not, for hemostasis?
10 DR. FEDERICI: The practical, the clinical
11 practice helps us. Not all of these patients treated with
12 Factor VIII concentrate correct the bleeding time but they
13 don't bleed. The other issue is Dr. White's questions. We
14 know for sure there is discrepancy between bleeding time and
15 ristocetin cofactor. The bleeding time -- you know, we have
16 people here who are very experienced, and Dr. Gralnick
17 showed very well in an old paper that the platelet content
18 is important as concerns the bleeding time. He showed very
19 well in the paper, in Blood, that you have a good
20 correlation between the bleeding time and von Willebrand
21 factor content, but you don't have the same correlation
22 between bleeding time and the plasma von Willebrand factor.
23 DR. RICK: Thank you.
24 DR. JOIST: Joist, St. Louis. I have a comment in

1 regard to the issue of the bleeding time. I think the
2 question here is are we measuring something, or are we
3 missing something measuring the bleeding time that is in the
4 von Willebrand factor concentrate or Factor VIII
5 concentrates rich in von Willebrand factor, or is it perhaps
6 that the bleeding time is influenced by something in vivo
7 that has nothing to do with the factor concentrates? For
8 instance, we know that there is a certain association of
9 congenital collagen vascular defects in patients with von
10 Willebrand disease.
11 So, it doesn't surprise me at all that there is
12 some variation where you have good ristocetin cofactor
13 levels, which really measure the concentrate activity in a
14 defined way, and the bleeding time does not correlate with
15 that ristocetin cofactor level. That has, to my knowledge,
16 not been adequately explored.
17 The second comment was in regard to the
18 questionnaire. I find it inconsistent that physicians or
19 treaters aim for 50-80% Factor VIII and, at the same time,
20 aim for 50-80% or ristocetin cofactor. You can't do that.
21 With a ratio of 2:1 to 4:1 that is just inherently
22 impossible. If they aim for 100% Factor VIII they, in fact,
23 acknowledge that they tolerate 300-400% von Willebrand
24 factor activity, which would worry me in middle age to

1 elderly patients. That is an issue that I think needs to be
2 looked at.
3 DR. COHEN: When people returned the questionnaire
4 there was a portion of returns that said we aim for the
5 ratio of 2:1, but then most people did not say that and they
6 just, you know, checked that they were doing both, which was
7 not well explained, why they were doing that.
8 What I got out of a lot of the survey was that
9 they were really monitoring this empirically; that they
10 would like this but then they weren't really testing and
11 were empirically managing these patients after the second
12 dose or so.
13 DR. KESSLER: Kessler, from Washington. I have
14 two questions. First of all, Dr. Federici mentioned that in
15 Italy they are using the Duke method for bleeding time.
16 DR. FEDERICI: Maybe I didn't explain. This was
17 the slide from Sweden. Maybe I didn't explain it very well.
18 The slide where you saw the Factor VIII C level more than
19 500 units is from a paper by Eric Bertorp, from Sweden,
20 published in Vox Sanguina. We don't do the Duke, we do the
21 Tech Simplate.
22 DR. KESSLER: Well, let me follow up again then
23 because some people believe that the Duke method may
24 actually be more sensitive for monitoring mucosal bleeds

1 rather than the modified IV which we use mainly in our
2 country. Have you seen this in your own clinical practice,
3 and do you feel that one bleeding time over another might be
4 able to reflect better intraplatelet content of von
5 Willebrand factor?

6 DR. FEDERICI: It is still an open question, but
7 we don't perform the Duke bleeding time so, as far as I
8 remember, in my center in the last twenty years I have
9 performed the Duke only a few times. So, we don't rely on
10 that. We still make diagnoses of vWD by using the Simplate
11 commercially available bleeding time.

12 DR. KESSLER: The other question that I wanted to
13 ask from the European perspective, and perhaps Dr. Lusher
14 asked this in her questionnaire and Dr. Federici has some
15 experience as well, is on the use of fibrin glue. In the
16 surgeries in von Willebrand patients it seems that the use
17 of fibrin glue might really overcome a lot of the
18 deficiencies and the unpredictability of maintaining
19 hemostasis in patients, and I was wondering if you would
20 comment on that, and Dr. Lusher as well.

21 DR. FEDERICI: We do use fibrin glue for dental
22 extraction mainly. The use of fibrin glue in more general
23 surgery is not so widespread in our surgery area. So, the
24 experience I have is that when we have -- actually, we are

1 collecting data by comparing in a population of von
2 Willebrand disease patients, sort of double blind, those
3 with severe type von Willebrand disease, those who go
4 through with prophylaxis and those who don't do prophylaxis
5 but just do fibrin glue. So, that means that when you have
6 local bleeding the fibrin glue can be helpful.
7 DR. SCHWARZ: I am Peter Schwarz, from Vienna.
8 Dr. Barrowcliffe gave us an excellent idea on the European
9 availability of von Willebrand factor concentrates. I would
10 just like to add that there seems to be inconsistencies
11 within the specific products regarding the labeling between
12 the different countries. If you look carefully into the
13 package inserts of those products, you will see that they
14 differ from country to country where the products are used.
15 However, in most of the countries those products are
16 indicated for replacement therapy of Factor VIII in patients
17 with von Willebrand disease who have decreased Factor VIII
18 levels.
19 What is interesting regarding the situation in the
20 U.K. is that there was a product available for the last
21 several years which had a label, as far as I remember, for
22 replacement of Factor VIII in other Factor VIII deficiencies
23 than hemophilia.
24 This is my remark on the European situation.

1 However, if I may add, I think the products made by the LFB,
2 the two different types, low Factor VIII containing von
3 Willebrand factor concentrate and the mixture, they seem to
4 have a very precisely and prospectively addressed package
5 insert, really outlined specifically for this disease that
6 we are talking about.
7 Concerning the assays, I am surprised not to have
8 heard anything about the use of platelet function analyzers
9 and if anybody wants to comment on that, I would be happy.
10 DR. MONTGOMERY: Montgomery, Milwaukee. I may not
11 be the best one to make this statement. Jim White makes a
12 much more impassioned point but, from his perspective and
13 many people's perspective, the bleeding time was never meant
14 to be a test of individual patients; it was meant to be a
15 population survey type of testing.
16 I think that in our own practice we probably do
17 still do it at diagnosis, and we don't know why. I mean, we
18 sit around and say, you know, if it agrees with what we
19 think then it is right, and if it doesn't then it is wrong,
20 not that we are wrong. So, we actually never use the
21 bleeding time in following patients in a therapeutic
22 situation, with the one exception that if there appears to
23 be continued hemostasis we will do that perhaps prior to
24 considering giving platelets as an additional therapy.

1 Then one other point on the problem with
2 insurance, while I don't want to beg the issue because it
3 certainly is a problem that has to be dealt with, but I have
4 found a trick around this, that if you diagnose von
5 Willebrand disease and Factor VIII deficiency it is at least
6 a way of using a Factor VIII concentrate to treat the Factor
7 VIII deficiency that is present in von Willebrand patients
8 and sometimes insurance companies can be convinced that,
9 therefore, it is an appropriate indication.
10 DR. RICK: Before you leave the microphone, let me
11 ask a question of you as well as the other speakers. Do you
12 think that if we treated to the point of normalizing a
13 bleeding time that we would be significantly over-treating
14 or treating just right?
15 DR. FEDERICI: It is not my data but, actually,
16 when I reviewed some of the important papers in the
17 literature -- in that slide I wanted to present on purpose,
18 you know, Eric Bertorp is very clever. So, he was
19 increasing the concentration of Factor VIII C/von Willebrand
20 factor concentrate in order to get a correction of the
21 bleeding time. If you go to the paper, this was a woman who
22 had ovarian section and was terribly bleeding. So he was
23 very concerned about correction of the bleeding time in vWD.
24 So he tried to give enough very high levels of von

1 Willebrand factor in order to keep within the Duke bleeding
2 time. So, we don't know because every patient can be
3 different. So, it is difficult to make a crossover in a
4 patient when you have an emergency.
5 That is as a general consideration, and back to
6 the comments by Peter Schwarz, you know, what is the content
7 of Factor VIII or should we use von Willebrand factor devoid
8 of Factor VIII, we actually don't know. That is why we want
9 to have this kind of European study to make a crossover in
10 the same patients to see how far we can go with one product
11 and how far we can have results with the other.
12 But the point is that when you go to the
13 hemophilia treaters, you know, they don't report failure by
14 the normal Factor VIII/von Willebrand factor concentrate.
15 So, one question they raised is why do you want to have
16 another product if these kind of products are working? So
17 this is a sort of general discussion but we have to produce
18 data about that. I think discussion is useful if we come up
19 with some conclusions at the end and we can start together
20 to figure out how we can come to the real solution.
21 DR. MAZURIER: Claudine Mazurier, France. I think
22 we could take the opportunity to ask a patient her opinion
23 about monitoring with bleeding time. So, I would ask Mrs.
24 McDonald if she would appreciate having the template

1 bleeding time.

2 MS. MCDONALD: Would I appreciate bleeding time?
3 No, I don't appreciate bleeding times at all. I had
4 multiple bleeding times when I was testing, and I do
5 appreciate the fact that you do need the data but my
6 prolonged time, when we sit there for 30 minutes, is very,
7 very boring and you are in enough pain after surgery and you
8 don't want the added stress of that. I don't like bleeding
9 times.

10 DR. MONTGOMERY: This is a secondary comment
11 because I have no direct experience with the platelet
12 function analyzers, but I do think that from the meeting
13 that was held in Florence what appeared to be evident is
14 that these are exquisitely sensitive to platelet von
15 Willebrand factor. Whereas, most of us probably expected
16 that this would be a real answer to the therapeutic problems
17 with von Willebrand disease, it is not because when patients
18 are treated with very adequate levels of von Willebrand
19 factor the closure time is still prolonged, and it really
20 appears that it is very exquisitely sensitive platelet von
21 Willebrand factor, particularly in patients with acquired
22 inhibitors where their plasma von Willebrand factor is very
23 low but their platelet von Willebrand factor would actually
24 be normal. Some of them have very normal closure times.

1 DR. WHITE: Well, I guess I am still not quite
2 sure whether bleeding time is overkill or not, Margaret, to
3 go back to your question. I think for most von Willebrand's
4 patients and for most procedures that are done or most
5 bleeding situations bleeding time definitely is overkill.
6 In our own center I think we try, in addition to a
7 diagnostic bleeding time when we diagnose von Willebrand's
8 disease, we probably do another bleeding time or two if we
9 are looking at a product to try and see if a patient will
10 respond to that product. When we initially give that
11 product we will often do a pre- and post-bleeding time to
12 ensure that there is correction with that product. For most
13 surgical cases we probably don't do bleeding times either
14 before or after treatment for surgery.
15 But I do think that there are only three tests
16 among all the tests that we can do that can be done in most
17 centers within an hour's period of time. That is, the
18 Factor VIII, the ristocetin cofactor and the bleeding time.
19 I think where one may say that one doesn't have to do a
20 bleeding time altogether, the problem with making a
21 statement like that is that that gets interpreted very
22 broadly as saying that a bleeding time is unnecessary and I
23 would be very hesitant to be in that situation. I do check
24 bleeding times sometimes in patients who are postoperative,

1 who are bleeding, in whom I am not sure whether I am getting
2 correction of both the Factor VIII and the ristocetin
3 cofactor activity.
4 So, my bottom line would be I don't think we have
5 the information to really answer your question right now. I
6 think we do in most cases but not in all cases. And I would
7 make an argument that we need to keep the bleeding time as a
8 viable test in certain situations in certain patients.
9 DR. RICK: Could I ask you one question about your
10 testing a patient's response to a product? If you do a pre-
11 treatment bleeding time and then a post-treatment time do
12 you insist that they be normal post-treatment, or is there
13 some measure of "significant" decrease that is satisfactory?
14 DR. WHITE: I don't mean to make light of the
15 question but I can't insist that a bleeding time be normal.
16 It is what it is. But, sure, I like to see it normalize but
17 it doesn't always do that. With some products it clearly
18 does. It depends, I think, in part upon how long the
19 bleeding time is to begin with. Clearly, with type 1 we see
20 complete time correction in the vast majority of cases.
21 Many of them don't even have bleeding time prolongation on
22 the pre-bleeding time. With type 3 it is much more
23 variable.
24 DR. RICK: What kind of dose are you using when

1 you see the complete correction?

2 DR. WHITE: You mean in a type 3 or a type 2?

3 DR. RICK: Yes, either, or more severe type 1.

4 DR. WHITE: Usually what we are doing in that
5 situation is a test dose for some procedure, maybe a minor
6 procedure, we are giving something that we would not
7 normally check a bleeding time for but we are checking
8 bleeding times for the future when they need more serious
9 surgery. So, we are typically giving just a standard dose.
10 I mean, we are giving a dose to raise the Factor VIII to
11 100%.

12 DR. RICK: The reason I asked the question is
13 because although there is certainly variability among the
14 concentrates as well as among the patients, it seems that
15 there is some dose response here, and I am wondering whether
16 we are significantly over-treating because we know that the
17 ratio of antigen is usually much higher relative to Factor
18 VIII activity in the concentrate.

19 DR. WHITE: Well, I think that is a good point. I
20 don't have any personal information on that because we
21 usually treat with a standard dose. Dr. Montgomery and I
22 were sitting next to each other when Dr. Federici presented
23 his work and we were interested that his levels of Factor
24 VIII were often in the 200 and 300 range. I wondered if

1 that was on purpose to get better bleeding time or
2 ristocetin cofactor correction, of if that was simply
3 related to bottle potency, or what. But I don't have any
4 personal information on dose-response curves to any of those
5 three parameters, other than Factor VIII.
6 DR. WEINSTEIN: I would like to bring up an
7 important point here that I think we have to set straight
8 for the record and for the purposes of this meeting. What
9 do people consider to be an overdose? What level would that
10 be, and what are the consequences, health consequences of a
11 so-called overdose? Is it more than financial? I would
12 like the physicians in the audience perhaps to respond to
13 that.
14 DR. MENACHE: I would like to make a comment. In
15 the study that we have done on type 3 vWD patients, using a
16 concentrate that does not contain Factor VIII, in our
17 pharmacokinetic studies we have found a correlation between
18 the levels of ristocetin cofactor activity and correction of
19 the bleeding time in patients with type 3. We only had ten
20 patients, but the ristocetin cofactor -- and I will show a
21 slide this afternoon -- had to be over 100% to get a
22 correction of the bleeding time. Again, all patients do not
23 correct. We had one outlier, as everybody else has.
24 DR. RICK: Thank you.

1 DR. FEDERICI: I want to answer. I don't have a
2 definite answer, of course, but this is just what I think
3 about the overdoses. It is really important to overcome the
4 defects of vWD, but how much we can correct the von
5 Willebrand factor antigen and, consequently the Factor VIII,
6 is not known.
7 But since I am a physician and I follow different
8 patients, other than vWD -- I am used to seeing patients
9 with cirrhosis for instance, and most of the people in the
10 audience know that cirrhosis is associated with high Factor
11 VIII C/von Willebrand factor complex. So, those patients
12 usually go through surgery and they can sometimes bleed
13 because they have a low platelet count but they have high
14 von Willebrand factor levels and high Factor VIII C. If you
15 go to measure Factor VIII C in those patients you have, I
16 suppose, more or less the same dosage that we have.
17 Of course, we have to be concerned about how much
18 von Willebrand factor and Factor VIII C we have in the
19 postsurgical patients because, you know, have Factor VIII
20 and von Willebrand factor are also important as factors that
21 are involved in cardiovascular disease. So, you don't want
22 to have a patient after surgery with so much von Willebrand
23 factor around. So, that is why we have to think about it.
24 Those case reports I presented were done, I

1 remember, by using a concentrate containing both von
2 Willebrand factor and Factor VIII by using between 50 and 80
3 U/kg. I think there is no definite recipe for von
4 Willebrand's disease. We have to think about it because
5 each patient can be a little different from another. So, we
6 have to make recommendations but we have to be a little
7 flexible and change this recommendation, first, according to
8 the type of patients and, most important, to the type of
9 bleeding episodes. So, this is something that we have to
10 think about during these discussions.
11 DR. BARROWCLIFFE: Could I just make a comment
12 about the concentrates used in the U.S. for treatment of von
13 Willebrand's disease? As Dr. Cohen mentioned, I think the
14 Alpha product, and these were described as solvent-detergent
15 intermediate-purity. I think that is not really quite
16 correct because they are, in fact, in relation to von
17 Willebrand factor relatively high purity but, perhaps more
18 important, the method of manufacture is the chromatography
19 process which is optimized for Factor VIII, which means in
20 practice that the von Willebrand factor content per unit of
21 8C would be different in those products and usually lower
22 than in the intermediate-purity products. This has,
23 obviously, consequences if, in fact, as we have already
24 heard the dosage for these products may be based on their 8C

1 content. So, in other words, you would be giving very
2 different amounts of von Willebrand factor in those products
3 compared to the intermediate-purity product, Haemate P for
4 instance.
5 DR. ARONSON: To talk to Margaret's question, as a
6 non-treater I have heard of almost as many cases of
7 thrombosis of von Willebrand's disease while under treatment
8 as have been reported in the literature of failures
9 resulting in hemostasis, over the last thirty years. I
10 think we are over-treating substantially.
11 DR. KESSLER: I wanted to address Dr. Weinstein's
12 question about the toxicity of Factor VIII. I think that
13 all of us as physician treaters differentiate between short-
14 term toxicity and long-term toxicity. I think that as far
15 as short-term toxicity is concerned, if we have to raise the
16 Factor VIII level of any individual with Factor VIII
17 deficiency of von Willebrand disease, I don't think any of
18 us are really concerned about the short-term toxicity of a
19 200% or 300% Factor VIII over a short period of time.
20 Now, perhaps there may be some long-term toxicity,
21 and I think some of the epidemiologic data that Ernest Briet
22 has reported indicating that maybe there is a correlation
23 between cardiovascular deaths and Factor VIII levels might
24 give us some pause on long-term toxicity if we felt that we

1 had to constantly maintain an elevated Factor VIII in a von
2 Willebrand patient.
3 But I think short-term, we don't really
4 replacement therapy to be toxic, at least I don't and if
5 others do here I would be interested in hearing their
6 comments.
7 DR. RICK: We are getting close to our time --
8 please, Dr. Federici, and then I would like to ask also if
9 Dr. Lusher has anything that she would like to add.
10 DR. FEDERICI: Just a very quick answer to this
11 problem. You know, in one of my case reports I presented
12 the case of the old lady, old, relatively old, 75 -- 75, so
13 she was not so old. This lady has a lot of problems,
14 hypothyroidism, hypertension and vWD type 2A. She has, as I
15 mentioned, prolonged bleeding time, more than thirty
16 minutes; no ristocetin cofactor activity, von Willebrand
17 factor antigen in their family, between 30-40% of antigen,
18 but Factor VIII C almost normal. So this was a real impact.
19 She had a heart attack. She had to have coronary-artery
20 bypass surgery. I discussed it with the surgeon because we
21 were facing two problems, a problem of achieving hemostasis
22 and not to have too much Factor VIII and von Willebrand
23 factor around. But this was sort of a challenge. So I want
24 to publish this data just because I want to maybe have some

1 comments from the reviewers for the future.

2 So, we have to deal with the fact that vWD

3 patients come late in their life because I believe, you

4 know, the von Willebrand factor is important for prevention

5 so the lack of von Willebrand factor can prevent having too

6 many thrombotic episodes but it is not the only factor for

7 arterial sclerosis.

8 So, the point is we managed this. We use Humate-P

9 in that case, and we know that we have to cover the bleeding

10 time factor without having too much Factor VIII C after the

11 operation, and we managed. I convinced the surgeon. I was

12 in there, and the real issue was we didn't do extracorporeal

13 circulation. The surgeon was so good that he was doing

14 bypass in the open heart. So this is one major point.

15 The second point was that we were in the operating

16 room. You can imagine, to convince to be there during the

17 operation to make the bleeding time more than 30 minutes,

18 and then give the Humate-P and then during the operation

19 make the bleeding time afterwards. So, I decided we will

20 treat the patient. I will stay in the operating room with

21 you and I will give more Humate-P if we see that the patient

22 is going to bleed. So, I know this is sort of a very

23 general and non-scientific way of treating but this was the

24 case.

1 DR. RICK: Thank you. I think many of us have
2 been in those situations. Dr. Lusher, would you care to add
3 to this discussion, especially to the bleeding time question
4 and correction?
5 DR. LUSHER: Well, I can't add much more than has
6 already been said. I think I agree with Dr. Kessler's
7 comments. Certainly for the short-term to raise the Factor
8 VIII level to 200% or above is not a major concern, whereas
9 it may be on a long-term basis.
10 As a pediatric hematologist, fortunately, I am not
11 faced with the erythematous plaques and so forth which some
12 of my adult colleagues are, and having used Humate-P for
13 many, many years for von Willebrand's patients, I have never
14 seen any thrombotic complication but that is probably, at
15 least in part, because of the age of the population I deal
16 with.
17 In that regard though, if patients are regarded as
18 being at risk because of those factors, it would seem that
19 the French product would be ideal in that it is a high
20 purity von Willebrand concentrate, the one that we will hear
21 more about later today.
22 In terms of bleeding times, I no longer use them
23 for monitoring any patient postoperatively or for a bleeding
24 problem, myself, and I remember a few years ago when we had

1 a von Willebrand three-day session at the Mayo clinic, a
2 symposium workshop, many people there pointed out the
3 anxiety that you can create in patients after giving them a
4 concentrate by doing a bleeding time preoperatively and it
5 is not corrected. It can create major emotional problems in
6 the patient and really doesn't add that much for the
7 transient shortening that one might see. So, we no longer
8 do them unless, as has been pointed out by others, there is
9 something unusual, or you suspect that you are giving enough
10 and, yet, the patient is still bleeding.
11 DR. RICK: Thank you. I would like to thank all
12 of the speakers and the participants from the audience. We
13 need to take a ten-minute break and return to the auditorium
14 then. Thank you.
15 (Brief recess)
16 **Assays for von Willebrand Factor**
17 **Potency Assays for von Willebrand Factor Concentrates**
18 DR. MONTGOMERY: I am Bob Montgomery. I am from
19 the Blood Center of Southeastern Wisconsin and Director of
20 the Blood Research Institute there. I have had a long-
21 standing interest in von Willebrand and it is nice to see
22 the issue of not having approved products to treat this
23 disease finally being addressed.
24 (Slide)

1 I would like to deal with potency issues. I am
2 not going to answer questions; I am going to bring up
3 problems, and that maybe is a recurring theme in dealing
4 with this but I want to show some data, some of which has
5 been seen in the past but that deal with some of the
6 problems with potency.
7 (Slide)
8 There are certainly a number of Factor VIII
9 concentrates that contain high levels of Factor VIII but
10 none of these concentrates are obviously labeled for the
11 level of vWF in these concentrates. These concentrates have
12 certainly been used successfully in the clinical management
13 of bleeding problems of patients with von Willebrand
14 disease. Thus, if asked the question are there successful
15 products right now to treat the condition, yes. Do we
16 always know what we are doing, no.
17 As recombinant Factor VIII products become either
18 the dominant or perhaps even exclusive accepted therapy for
19 treating hemophilia A, the plasma-derived combination
20 products that contain both proteins may have their primary
21 use or even exclusive use as an off-label treatment of von
22 Willebrand disease and, thus, I think drive some of the
23 desire to do something about this prior to the time, I am
24 sure the FDA wouldn't be real comfortable with the

1 predominant use of a product being an off-label use.
2 Purified plasma-derived von Willebrand factor
3 concentrates and recombinant von Willebrand factors are also
4 being developed as specific therapy for von Willebrand
5 disease. I think that the issues are how can consistency of
6 manufactured products be assured, and how should dosing of
7 patients be determined, and what is the scientific basis for
8 dosing, and how should clinical efficacy be determined? I
9 don't have anything to offer on the latter ones but are
10 certainly ones that we will be discussing through the day.
11 (Slide)
12 What really is the problem? Unlike Factor VIII,
13 von Willebrand factor is a multimeric protein. So, not only
14 can you have problems with the amount of protein, the
15 activity of that protein but, on top of that, it is
16 confounded by the structure of that protein. I think
17 because of that, it is a unique problem.
18 There is no accepted in vitro method that assays
19 the in vivo activity of von Willebrand factor. Antigen
20 assays do not reflect activity but are probably most easily
21 standardized.
22 Ristocetin cofactor assays can be inconsistent if
23 assaying purified proteins. I think that with standard
24 assays for ristocetin cofactor there can be good agreement.

1 The problem is that how people do ristocetin cofactors are
2 quite varied. Whether one uses even platelet counting after
3 the addition of ristocetin, the visual interpretation of the
4 snowstorm that occurs with agglutination, the laboratory
5 measurement of the slope of aggregation or agglutination,
6 more properly, or the absolute amount of agglutination, all
7 are different parameters that are used.
8 Multimeric determination reflects the quality but
9 the significance, actually, at the highest level is
10 debatable. I think everybody would like to give the most
11 normal appearing vWF multimer protein but, in fact, whether
12 there is a difference between the material that is at the
13 very highest point of the multimeric analysis or maybe down
14 a couple of multimers from that is not fully clear.
15 Collagen binding may not always reflect the
16 biologic activity but it is relatively consistent. By that,
17 I mean that there are variants of von Willebrand protein
18 that vary in their binding to collagen, yet, the activity
19 once bound to collagen may, in fact, be normal.
20 Platelet function analyzers or similar devices do
21 not reflect the biologic improvement following therapy and
22 may, actually, be a better measurement of platelet von
23 Willebrand factor content.
24 I think the bottom line is that concentrates need

1 to be labeled in the units used to dose the patient and that
2 the patients will be followed.
3 (Slide)
4 Standard assays for analyses of von Willebrand
5 factor include antigen and there certainly are differences
6 with ELISA and quantitative immunoelectrophoresis or Laurell
7 technique. Certainly, there are differences in the way
8 ristocetin cofactor assays are being done, but in general
9 the relationship between antigen and ristocetin cofactor
10 should be 1:1. I think that there are problems that I will
11 mention in a subsequent slide about that.
12 Looking at vWF are multimers and then one that we
13 don't have any direct experience with ourselves, except in
14 looking at vWF variances, is collagen binding. For example,
15 2B von Willebrand factor may, in fact bind to collagen but
16 once it is bound it has no increased activity in contrast to
17 what it is in fluid phase.
18 (Slide)
19 Just to point out different ways, this points out
20 what was described earlier, that is, the importance of
21 differences in how multimeric determinations are done, and
22 whether one amplifies the differences when you call this a
23 low resolution gel when, in this case, it is a high
24 resolution for what you are trying to study, which is the

1 presence of high molecular weight multimers. But,
2 certainly, to use an assay that is reflective of the
3 reproducibility of the manufacture may be important.
4 (Slide)
5 This only deals with the relationship. We have
6 actually found correlation coefficients of antigen and
7 ristocetin cofactor in excess of 0.96. Even that
8 correlation holds true in patients with von Willebrand
9 factors less than 50. This happens to be 690 patients, in
10 the yellow box, that actually are studied and you can see
11 how tightly these cluster around a ratio of 1 or ristocetin
12 to antigen. So, for most practical purposes these two
13 things are correlated at least with normal von Willebrand
14 factor.
15 (Slide)
16 Just to point out, not going to a lot of detail,
17 but the assay that we will be doing and that I will be
18 discussing is using a ristocetin cofactor assay where the
19 slope of the agglutination curve is in fact what is used to
20 determine the amount. We are using formalin-fixed platelets
21 that are made in-house, which have a much stronger
22 agglutination response than the commercial fixed platelets
23 that are available, but certainly standardization is a
24 problem. In dealing with the assay for vWF concentrates, we

1 would assume that dosing would be used by units of Factor
2 VIII currently but, certainly, this is not an ideal
3 situation but as far as looking at concentrates we, in fact,
4 did our initial concentration based upon Factor VIII units.
5 Then we assayed these concentrates in buffer, in 0.5% or 1%
6 BSA, and then in severe von Willebrand plasma by making the
7 sample equal to 100% based upon the labeled Factor VIII
8 units. Then we assayed in units of ristocetin cofactor and
9 von Willebrand antigen, did multimeric determination and
10 then as a side of this, looking at the question of
11 commercial standards that are used for vWF.
12 (Slide)
13 Now, why we got into this -- and this is merely a
14 representation slide; it is not meant to be precise data but
15 it is why we got into this, having to deal with what I
16 consider -- actually, in the last issue of Blood Dominique
17 Meyer's group is again trying to address the question of
18 GP1B on endothelial cells. But we got into this because
19 early on we also were studying the interaction of von
20 Willebrand factor with endothelial cells.
21 What you can see here is that in the presence of
22 ristocetin that von Willebrand factor binds to endothelial
23 cells. In fact, if you use AP1, which is a monoclonal
24 antibody to GP1B, you inhibit that binding. If you use a

1 monoclonal antibody to von Willebrand factor ABW3, not shown
2 here, you see the same thing. So it appears that if you
3 inhibit the vWF binding to GP1B, either by antibodies to 1B
4 or to vWF, you block this binding so everything seems fine.
5 That was until we did additional experiments and
6 we used AP2, which is an antibody to 2B3A, not on
7 endothelial cells. It blocked it. And if we used
8 irrelevant IgG, adding it to the material, it also blocked
9 it. If we looked at lymphocytes we found von Willebrand
10 factor binding to those lymphocytes. As it turns out, if
11 you add albumin or if you add other proteins you will block
12 this binding, and that is because -- in papers that were in
13 the JBC and elsewhere -- ristocetin precipitates proteins,
14 particularly when they are purified.
15 (Slide)
16 So, in order to look at this, if you take just
17 purified von Willebrand factor and then just add ristocetin,
18 you find here about 40% of the von Willebrand factor in fact
19 precipitates. As you add albumin you actually markedly
20 decrease this and, in fact, at even higher concentrations
21 you can almost totally block it. Why? Because other plasma
22 proteins are competitors for the presence of ristocetin on
23 them, particularly fibrinogen and others.
24 How does this affect an assay? If you have

1 purified von Willebrand factor and add it to platelets and
2 add ristocetin and you have a precipitation occurring and
3 you look at binding, then you see an increase apparently.
4 But if you do the same experiment and look at agglutination,
5 the precipitation removes von Willebrand factor from it and,
6 therefore, the assay will be lower.
7 (Slide)
8 So, we looked at a number of concentrates,
9 identified here just by letters, and they in general are
10 ranked, with the exception of this one, according to the way
11 their multimers would appear in general. So, the one at the
12 top would have the more normal looking multimers.
13 Then we assayed these and they were diluted to be
14 100% Factor VIII. They then had the ristocetin cofactor and
15 antigen. As you can see, there is more von Willebrand
16 factor than the amount of Factor VIII in most of them, not
17 in all. But if you look at the specific activity, and this
18 column will always be the ratio of ristocetin cofactor/unit
19 antigen, but you can see that they vary greatly. In
20 general, these will be lower than the other assays that I
21 show you. Why? Because this is purified von Willebrand
22 factor in buffer in which there is undoubtedly precipitation
23 that is preventing the von Willebrand factor from
24 agglutinating those platelets.

1 (Slide)

2 If you do this in albumin then you see similar
3 things. On this one we have added another concentrate that
4 has very little Factor VIII in it, and you can see that the
5 levels of von Willebrand factor, both antigen and activity,
6 increased. The specific activity, you can see, varies.

7 (Slide)

8 I want to spend most of my time on the final slide
9 which has to do with when we reproduced the clinical
10 situation. When w infuse patients with von Willebrand
11 factor we are putting it essentially into 100% plasma. So
12 it seems like we ought to adopt a similar way of approach
13 that we do for hemophilia assays, which is to reconstitute
14 concentrates in the deficient plasmas prior to assay.

15 What is pointed out here is, as you can see, that
16 there may be a 3-fold or greater amount of ristocetin or
17 antigen compared to the Factor VIII. So, if we use Factor
18 VIII units we are going to be over-treating patients by a
19 factor of 3. But also, the specific activity of
20 concentrates varies greatly. You can see that if we take
21 this concentrate, which is no longer even on the market, the
22 ratio of the ristocetin cofactor to amount of antigen is
23 about 1/4 to 1/5 the level seen in perhaps the highest
24 concentrate.

1 (Slide)

2 If we look at multimers you can see that they vary
3 greatly. This gel, you can see, has a setup so that it
4 emphasizes differences at the high end, and you can see that
5 there is a marked difference in concentrates as far as their
6 multimeric appearance.

7 I have been asked to chair a subcommittee to deal
8 with the laboratory diagnosis of von Willebrand disease for
9 the ISTH, but there is a problem that is not just in
10 concentrates because we have had a number of centers that
11 have sent their samples to our referral laboratory and I got
12 this call one day that said, "I think you'd be interested
13 from a molecular standpoint in studying what's a most common
14 variant that our laboratory sees, in which the ristocetin
15 cofactor activity is higher than the antigen." And I said,
16 "really? Let me see one of those." So they sent us a
17 sample and we assayed it, and I think it was 81% ristocetin
18 and 85% antigen, right on.

19 So I said, now send me your standard. And they
20 sent us their standard, and their standard assayed out with
21 a low ristocetin cofactor activity compared to the amount of
22 antigen. So, as soon as you reference against that your
23 normal individual will, in fact, have higher ristocetin
24 cofactor activity than the amount of antigen. This was

1 caused by proteolysis of von Willebrand factor that, at
2 least with the Laurell Rocket, will increase the amount of
3 antigen at the same time that it decreases the ristocetin
4 cofactor.
5 (Slide)
6 This is more recent, just trying to look at a
7 number of commercial standards. When we do studies we do
8 reference against the World Health Organization reference
9 point. You can see that we in general do not agree with the
10 absolute level, and I would say that when we survey randomly
11 100 patients we find that if we use the World Health
12 Organization standard our mean level is actually between 115
13 and 120. So at least in the population that we deal with,
14 we find that the WHO standard is a little bit low. But that
15 is not necessarily the critical issue because the critical
16 issue is that we have a standard reference point and,
17 certainly, the WHO standard offers us that.
18 But you can see that a number of the standards
19 have abnormal multimers. In this particular one the
20 specific activities actually look relatively good, but you
21 can see that if you use the WHO standard for ristocetin
22 cofactor, there are standards that claim to be referenced
23 against it that are in excess of 2-fold greater than it and
24 obviously can cause a problem. So, much of the problem

1 between laboratories may not just be the assays, it may also
2 be the reference standards.
3 (Slide)
4 This is a very preliminary single experiment but I
5 think it also points out some interesting things. Disregard
6 the data on this, I am only wanting to call your attention
7 to the fact that this is a shear chamber that Larry
8 McIntire's group has developed and that we are utilizing to
9 look at shear-induced binding of platelets to collagen in
10 the presence of von Willebrand factor.
11 (Slide)
12 Just to point out that this follows much of the
13 things that are known about this. We are looking here at
14 platelets bound per square millimeter. If you look at a
15 control with whole blood, you get this amount of binding.
16 If you block through antibodies to GP1B, you block
17 antibodies to von Willebrand factor, you block that.
18 Actually, antibodies to 2B3A show a participation in the
19 finalization of really the fixation of platelets to the
20 surface. This is AP2. It blocks that. Then if we lower
21 shear rates and use intermediate shear we actually get a
22 reduction.
23 (Slide)
24 I will point out at the beginning that this is a

1 single experiment so there is a real problem with this data
2 point, but what I want to point out is that this is taking
3 whole blood from a patient with type 3 von Willebrand
4 disease and then adding a commercial concentrate to it. So,
5 you can see that when you add the commercial concentrate you
6 have a marked improvement in the platelet binding, in fact
7 in excess of the control, and you see a fall-off and
8 somewhere between 40% and 10% there is a marked diminution.
9 Obviously, here platelet von Willebrand factor content is
10 zero.
11 So, I think there are ways of being able to study
12 an in vitro correlate, and I would point out the importance
13 of maybe looking at some of these along with the study of
14 new clinical concentrates.
15 (Slide)
16 Certainly a consensus must be reached of both
17 physicians and industry on what the right assay is that must
18 be standardizable and reproducible; must reflect consistency
19 of production; and must determine what the reference
20 standard should be. The assay must reflect dosing so that
21 one has some idea from the vial what you are going to be
22 using in the patient. The in vivo recovery must be done in
23 the same units as labeled potency.
24 Recommendations, from my perspective, would be

1 that ristocetin cofactor needs to be done in a standardized
2 manner because it is widely used, the results are relatively
3 quick and it is probably the most accepted assay for vWF
4 activity.
5 There would have to be the ability to pre-dilute
6 the reconstituted concentrate in either type 3 von
7 Willebrand plasma or perhaps immunodepleted normal plasma,
8 and then to use specific activity as the measure of
9 production consistency so that the amount of ristocetin
10 cofactor or collagen binding to antigen would, in fact, I
11 think carry with it the assessment of at least adequate
12 multimers because I think that multimeric quantification is
13 more problematic.
14 There may be a problem, and this has to do with
15 the fact that as recombinant von Willebrand factor -- or if
16 you look even further down the road, mutant molecules of von
17 Willebrand factor that have preferred activity, whatever
18 that preferred activity is, may, in fact, not have a 1:1
19 relationship between activity and antigen, yet, could still
20 be used to reflect the manufacturer consistency.
21 We really need to point out what the clinicians in
22 the audience know about the problems with B-domainless
23 Factor VIII, and it is causing people to reexamine do you
24 dose people based upon the number of Factor VIII units, or a

1 particular clotting assay that is done <u>in vivo</u> to assess the
2 infusion of that product?
3 I think we just have to keep an open mind the fact
4 that there may be materials that are perfectly valid for
5 treating patients that may have a relationship between
6 antigen and activity other than 1:1, that may help us even
7 address some of the issues of dosing as we get into this
8 period of time.
9 So, I think with that, I will finish up and I will
10 move on to the other speakers. If we have time at the end,
11 although no time was specifically allocated, we can take
12 questions together at the end.
13 What I would now like to do is to introduce the
14 second speaker of this session, Dr. Anthony Hubbard who is a
15 principal scientist in the Division of Hematology at the
16 National Institutes for Biologic Standards and Control, in
17 the U.K. Dr. Hubbard?
18 **Assay for von Willebrand Factor in Factor VIII Concentrates**
19 DR. HUBBARD: Good morning, ladies and gentlemen.
20 (Slide)
21 I would like to present some data on a preliminary
22 study carried out at NIBSC on the assay of von Willebrand
23 factor and various Factor VIII concentrates.
24 (Slide)

1 As regards NIBSC, the two most important points,
2 obviously, are the control and standardization of vWF and
3 Factor VIII concentrates, and by control I really mean we
4 need to agree on what parameter we use to label the products
5 which can be used by all manufacturers so that we can
6 compare the dosage of different products. For this to be
7 meaningful, obviously the parameter has to relate to
8 clinical effectiveness. Hand in hand with control comes the
9 standardization of measurement of vWF. We need to ask
10 ourselves if we can use an existing plasma vWF or whether we
11 need a specific concentrate standard.
12 We also need to look at the inter-laboratory
13 variability of vWF assays and Factor VIII concentrates.
14 There is no point in agreeing which parameter to measure if
15 there is excessive variability between laboratories. In
16 fact, as mentioned earlier, it was shown, in a publication
17 in 1993 by Fricke et al., that there can be an excess of a
18 2-fold variability in von Willebrand factor estimates when
19 laboratories measure the same samples.
20 (Slide)
21 The preliminary study we carried out really had
22 two objectives, firstly, to asses the validity of assaying
23 Factor VIII concentrates versus a plasma standard and we
24 chose to use a straightforward antigen measurement and

1 collagen binding measurement. We also wanted to compare the
2 content of antigen and collagen binding in different
3 products.
4 (Slide)
5 The measurement of antigen with conventional ELISA
6 using commercial antibodies, the catching antibody and the
7 detector antibody were both polyclonal rabbit antihuman vWF
8 antibodies. Detection was using a horseradish peroxidase
9 conjugate.
10 (Slide)
11 For collagen binding we used an assay based on the
12 original paper of Brown and Bosak, except that we used a
13 different source of collagen. The detecting antibody was
14 the same detector used in the previous assay. All test
15 sample dilutions in both assays were carried out using a
16 buffer containing 1% human albumin.
17 (Slide)
18 For the assay design and potency estimation we
19 wanted to obtain as much information as possible from these
20 assays. So, we assayed at least three dilutions of test and
21 standard, and each dilution was tested at least in replicate
22 in each assay. This allows us to construct a dose-response
23 curve for both standard and test, which gives us valuable
24 information regarding assay validity.

1 Potency was estimated versus a calibrated British
2 standard plasma, which was itself directly calibrated
3 against the WHO international standard. As you probably
4 realize, the WHO international standard is only calibrated
5 for vWF antigen and ristocetin cofactor. Therefore, the
6 potencies for the collagen binding assay were calculated
7 using the assigned potency for ristocetin cofactor.
8 Calculation of the potencies was carried out using
9 parallel line analysis techniques which compare the log dose
10 response relationships. We used the log-log transformation
11 since this gave us the best linearity for dose response.
12 (Slide)
13 This is an example from one of the assays we
14 carried out. This was vWF antigen assay on a batch of
15 concentrate A. Here we have the four dilutions of the
16 plasma standard and four dilutions of the concentrate
17 materia. Each dilution was assayed using four replicates in
18 this case. We have a mean and a range of values plotted
19 here on a log-log scale which gave us a nice linear
20 response. The computer program we use at NIBSC gives us a
21 measure of validity by the linearity and the parallelism of
22 these two dose-response relationships. The potency of
23 concentrate A is calculated using the horizontal distance
24 between the two dose-response lines.

1 (Slide)

2 The test samples we looked at were four Factor
3 VIII concentrates. Two are intermediate purity, prepared
4 using conventional precipitation techniques and these have
5 been coded A and B. We also looked at two high purity
6 concentrates which were prepared using chromatographic
7 techniques, coded C and D. Only concentrates A, B and C
8 have been used for von Willebrand's disease therapy.
9 Concentrate D has never been proposed, and probably never
10 will be proposed for this therapy but it was included since
11 it was manufactured using similar technology to concentrate
12 C.

13 We also looked at a few plasma samples, mainly for
14 control reasons, just to look at the behavior of the assays,
15 the international standard and two British standards. These
16 are actually pooled freeze-dried plasmas. We also looked at
17 two commercially available Factor VIII-deficient plasmas.

18 (Slide)

19 These are the results from the control assays on
20 the plasmas, the vWF antigen and the collagen binding
21 results in U/ml. Basically, there is very good agreement
22 between the two assay methods with the pooled freeze-dried
23 plasmas, as you might expect given that the ratio is very
24 similar to 1. Also, with one of the Factor VIII-deficient

1 plasmas which was chemically depleted, this also gave us a
2 ratio close to 1. An immunodepleted Factor VIII-deficient
3 plasma showed slightly reduced collagen binding, which might
4 indicate slight denaturation during the manufacture.
5 (Slide)
6 If we note the results on the concentrates,
7 basically they express the antigen and collagen binding per
8 unit of Factor VIII C, and we also looked at the ratio of
9 collagen binding to antigen.
10 (Slide)
11 This slide shows the antigen results for the four
12 concentrates. You can see the ratios here. This is the
13 mean value from the numbers of batches that were tested, six
14 batches of concentrate A and three batches of concentrate B,
15 C and D. We have a mean value and the extreme range of
16 values. So, within each product we had quite good agreement
17 in the vWF antigen, a wide range here. Of course, all
18 ratios are greater than 1, but a wide range within the
19 products, greater than 2-fold between concentrates B and C,
20 with concentrate B obviously containing much larger amounts.
21 Concentrate D, which has never been used for therapy -- you
22 can probably see why, it contains very little antigen.
23 (Slide)
24 Looking at the collagen binding, we have a similar

1 profile but not exactly the same. Again, we have the
2 largest amount of collagen binding for concentrate B and the
3 smallest amount for concentrate C, if you look at the three
4 materials used therapeutically, approaching a 3-fold
5 difference between concentrates B and C and around 2-fold
6 for concentrates A and B so a wide range there.
7 (Slide)
8 If we look at the ratio of collagen binding to von
9 Willebrand factor antigen, again we see a fairly wide range
10 with concentrate A giving the lowest ratio. These
11 differences probably reflect different degrees of
12 denaturation during the manufacture. So, concentrate B not
13 only contains the highest amounts of vWF antigen but it
14 appears to be the least denatured as well.
15 (Slide)
16 We plotted all of the results from the collagen
17 binding assays and the antigen assays for the four
18 concentrates, and you can see there is a fairly good
19 correlation despite the ratio differences within the
20 product.
21 (Slide)
22 Looking at assay validity and precision, the
23 validity was examined by comparing the parallelism of the
24 log dose response relationships between the concentrates and

1 the standard. Inter-assay variability was looked at by
2 calculating the coefficient of variation from the peak
3 assays, and intra-assay precision was investigated by
4 looking at the 95% confidence limits with each individual
5 assay.
6 (Slide)
7 This slide looks at the parallelism of the dose-
8 response relationship between the test concentrates and the
9 plasma standard. In this slide the slopes of the test
10 concentrates are being plotted as a percentage of the slope
11 of the plasma standard. So, 100% indicates complete
12 parallelism.
13 First, I would like to point out that within each
14 product the results are very tight except, of course, for
15 the antigen measurements on concentrate A. In fact,
16 statistically there are significant differences between all
17 of these assays and the slope of the plasma standard, except
18 for the antigen assays here and the collagen binding for C
19 and D. So, basically this dotted line here represents 85%
20 agreement in the slope. So, most of these concentrates are
21 around this range or above, which indicates that we can
22 obtain, with some confidence, a good potency estimate for
23 all of these assays but what is interesting is that there is
24 an obvious trend for all of these assays to have slopes

1 slightly less steep than the plasma standard. This probably
2 might be an indication that we should be looking at possible
3 concentrate candidate standards or even some modification to
4 the assay, such as a predilution of the concentrates in von
5 Willebrand factor deficient plasma.
6 I don't want to be too pessimistic because we can
7 still obtain good potency estimates using this system, and
8 if we look at the most non-parallel assay from this study,
9 which was this one here for the antigen measurement on
10 concentrate B, it is quite obvious that there is a non-
11 parallelism between the two dose-response lines of the
12 concentrate and the standard. However, the degree of
13 overlap is such that we can still, with some confidence,
14 obtain a relative potency for concentrate B. So, I am not
15 saying that all is lost using the present system of
16 concentrate versus plasma, but I think there is some room
17 for improvement.
18 (Slide)
19 Looking at inter-assay variability, we have only
20 carried out four repeat assays using each method, on this
21 slide. In this case we looked at the European Pharmacopoeia
22 Factor VIII concentrate standard and we compared this to the
23 British standard plasma. The bottom line is that the
24 coefficient of variation was 6.3% for the antigen

1 measurement and 8.5% for the collagen binding, which is
2 quite respectable when we are only looking at such a small
3 number of assays.
4 (Slide)
5 Precision of potency estimates was looked at by
6 converting the 95% confidence limits from the individual
7 assays as a percentage of the mean potency from the
8 individual assays. For the antigen measurements, basically
9 we are looking at a range of plus/minus 10% of the mean, and
10 this is even tighter for the collagen binding measurements.
11 So, both assay methods show fairly good intra-assay
12 precision.
13 (Slide)
14 To conclude, the different products varied widely
15 in the ratios of antigen to HC, collagen binding to and
16 collagen binding to antigen. So, therefore, dosage on the
17 basis of HC or antigen would lead to the infusion of
18 different amounts of functional activity and this would have
19 implications in comparing the clinical effectiveness of
20 different products.
21 (Slide)
22 Just to emphasize this point, we have recalculated
23 what would be the total content of vWF in, say, a typical
24 500 IU vial of the three products used therapeutically. You

1 can see that dosage on the basis of HC or antigen would lead
2 to greatly varying amounts of collagen binding or vWF
3 function being infused.
4 (Slide)
5 There is a trend for non-parallelism between the
6 Factor VIII concentrates and the plasma standard, with the
7 plasma standard us equal log dose response relationship.
8 Further studies are required to look at the effect of
9 prediluting the concentrates in vWF-deficient plasma and to
10 investigate parallelism with potential concentrate
11 standards.
12 (Slide)
13 The collagen binding assay is easy to perform and
14 gives a precise estimate of vWF function. However, adoption
15 for labeling purposes requires conclusive evidence of
16 clinical relevance and an investigation into inter-
17 laboratory variability and methodology. We need to know if
18 the source of collagen would have an effect on this assay,
19 and also whether the predilution in vWF-deficient plasma
20 would also have an effect. Thank you.
21 DR. MONTGOMERY: Our third speaker will be Dr.
22 Peter Turecek, who is head of the Research Division and
23 Product and Process Development of Blood Coagulation at
24 Immuno AG, in Vienna, and has done a great deal of work with

1 the collagen binding assay both as it applies to plasma as
2 well as recombinant von Willebrand factor.

3 **The Determination of von Willebrand Factor Activity**
4 **by Collagen Binding Assay**

5 DR. TURECEK: Dr. Montgomery, ladies and
6 gentlemen, first of all I would like to thank Dr. Weinstein
7 for giving us the opportunity to present here our
8 perspective on a quantitative assay for the function of von
9 Willebrand factor.

10 (Slide)

11 This is almost the same slide as Dr. Montgomery
12 showed you before, just giving you a brief introduction of
13 what I want to talk about. These are the routine assays
14 which can be carried out for measuring von Willebrand factor
15 antigen, multimer analysis, Factor VIII binding, ristocetin
16 cofactor activity and collagen binding. These are the
17 assays which are most used routinely. There are a variety
18 of other assays at present measuring interaction of GPIB,
19 RIPA and so on.

20 (Slide)

21 The reason why we are in favor of the collagen
22 binding assay is that the binding epitopes have been mapped.
23 They are located in the A1 and A3 domain of the mature
24 subunit of the von Willebrand factor molecule.

1 (Slide)

2 Dr. Hubbard, the previous speaker, has already
3 addressed the collagen binding assay very carefully. What I
4 am showing you here now is almost the same, an assay where
5 collagen is attached to a microtiter plate which interacts
6 with von Willebrand factor and is then detected with a
7 polyclonal antibody which is peroxidase labeled.

8 So you may ask yourself what is now the difference
9 of the special thing of this assay. There are three points.
10 One is the type of collagen we used. This is a type 3
11 collagen from human placenta which has been pepsin-digested,
12 which is covalently -- this is the second point -- mobilized
13 to the microtiter plate.

14 (Slide)

15 The third point is that the concentration for
16 coating the microtiter plates in this assay is much lower
17 than what is used routinely. Most other published collagen
18 binding assays for von Willebrand factor assessment require
19 concentrations in the range of 30 mcg/ml collagen, and the
20 collagen we use is sufficient to have in a concentration of
21 around 3 mcg/ml.

22 Why is this of importance? I should remind you
23 that collagen is not a soluble protein. Due to its unique
24 amino acid sequence and structure with repetitive epitopes

1 and its capability to interact with interchains of the
2 molecule, it has a tendency to form fibrins. This is
3 crucial when you coat the microtiter plate with a protein
4 which has a tendency to form certain three-dimensional
5 structures. You have to avoid this in general. The way we
6 have solved the difficulties in making reproducibly coated
7 microtiter plates is to utilize pepsin or protease-digested
8 collagen on the one hand, and on the other hand, a rather
9 low concentration which is more in the range of the coating
10 conditions for immunosorbents. In normal ELISA technology
11 immunoglobulins are utilized in a concentration of 1 or up
12 to 5 mcg/ml for coating, but not in the high range, as
13 published for the collagen binding assays which are
14 appearing in the literature.
15 The third point, and I have mentioned this before,
16 is that the collagen is covalently bound to the surface,
17 which allows a very consistent and highly reproducible
18 appearance of the active surface in this assay.
19 (Slide)
20 The assay is really specific for von Willebrand
21 factor. In von Willebrand factor deficient plasma from a
22 type 3 patient there is almost no collagen binding activity
23 measurable. If we reconstitute recombinant von Willebrand
24 factor in buffer or in von Willebrand factor-deficient

1 plasma -- we did this in a concentration of about 1.6 U/ml,
2 we had 100% recovery in both buffer and the deficient
3 plasma. There is obviously no interaction with any other
4 plasma protein in the deficient plasma sample.
5 (Slide)
6 Calibration curves are looking very good. If we
7 want to assess concentrates we would normally have to dilute
8 our samples anyway, so in this range we have very good
9 calibration curves. The limit of detection of this assay is
10 even lower. It is in the range of around 10 ng/ml. The
11 limit of quantitation is around 20 ng/ml, although I have
12 here started this calibration curve at 100 ng/ml.
13 (Slide)
14 The stability of the precoated microtiter plates
15 is shown in this slide. This is again the absorbance of the
16 calibration curves with increasing von Willebrand factor
17 amount. Storage at 37 degrees for 5 weeks does not harm the
18 calibration curve of this assay.
19 (Slide)
20 The inter-assay reproducibility with plasma-
21 derived Factor VIII/von Willebrand factor concentrate --
22 this is our in-house standard for Factor VIII/von Willebrand
23 factor concentrate. It is in an acceptable range. The
24 coefficient of variation is not written here but it is below

1 10%. It is quite similar to what Dr. Hubbard has shown us
2 before. It is around 8.5% or 8.3%.
3 In comparison, we make aggregometric ristocetin
4 cofactor activity assay for the von Willebrand factor
5 activity in our concentrate. The coefficient of variation
6 is here in the range of about 15%.
7 (Slide)
8 Collagen binding correlates with ristocetin
9 cofactor activity. Here we measured 29 normal plasmas from
10 healthy donors. The correlation from our perspective was
11 acceptable. Most of the normals have a ratio of 1:1.
12 (Slide)
13 The collagen binding activity also correlates with
14 the appearance of the von Willebrand factor multimers. In
15 this experiment we have degraded a recombinant von
16 Willebrand factor preparation with the depolymerizing
17 protease, the protease which has been described both by the
18 groups in Switzerland and the United States, Fullan and
19 Tsai. This polymerase cleaves at the tyrosine methionine 8,
20 42, 8, 43 position of the von Willebrand factor subunit and
21 causes the proteolytic degradation which then appears the
22 triplet structure of the protein. We have measured here a
23 time-dependent degradation of the von Willebrand factor
24 molecule. You can see that the high molecular weight

1 multimers disappear, and in the same range as the multimers
2 disappear the collagen binding activity, the red line, goes
3 down to almost zero. The arrows here should indicate that
4 the recombinant von Willebrand factor then develops the
5 typical triplet structure, but I think this is addressed in
6 another lecture by Dr. Schwarz at the end of this day in
7 more detail.
8 (Slide)
9 I can skip this slide because you are all aware of
10 the von Willebrand factor subtypes.
11 (Slide)
12 This is a picture which I have modified a little
13 in that I have now added collagen binding activity which
14 correlates to the ristocetin cofactor activity in various
15 subtypes of von Willebrand's disease. In type 1 it is
16 decreased; in type 2 it is also decreased or even normal; in
17 type 3 it is markedly decreased.
18 (Slide)
19 We had the opportunity to participate in a study
20 together with a group of Dr. Blanchette, in Toronto, where
21 we used this assay, together with other von Willebrand
22 factor assays, to go through all his patient samples, plasma
23 samples. I have listed here a few examples of what we found
24 in this group.

1 Patient F, for example, is a typical type 3
2 patient, no multimers, the antigen is below the limit of
3 detection and even ristocetin cofactor and collagen binding
4 is below the limit of detection. But we even found patients
5 where we had a slight response in the antigen assay,
6 although on the multimer gel no von Willebrand factor was
7 visible. In this case it really helped to have the collagen
8 binding assay because here we also could prove that there is
9 no functional activity of von Willebrand factor left in this
10 patient.

11 We heard before from Dr. Cohen that multimer
12 analysis is not used frequently for assessing von Willebrand
13 factor subtypes or patient analysis, and you forget about
14 the numbers here in the line where the multimers are written
15 and look at patient B. This also has the von Willebrand
16 factor antigen level at the limit of detection or slightly
17 above the limit of detection but no ristocetin cofactor
18 activity. If you want to know that multimers are present in
19 this patient you could even subtype this to a type 3
20 patient. But if you do the collagen binding assay, which is
21 more sensitive than the ristocetin cofactor method, you see
22 that there is functional activity left in the patient and
23 now it was clear that patient B was a subtype 1 von
24 Willebrand.

1 (Slide)

2 This is an experiment of an infusion of a plasma-
3 derived von Willebrand factor concentrate, Immunate, where
4 46 ristocetin cofactor units/kilo body weight were applied,
5 and we measured the pharmacokinetics in this patient. We
6 see that ristocetin cofactor, the red curve, and collagen
7 binding curves fit rather well. The PK both in collagen
8 binding and ristocetin cofactor activity are almost similar.

9 (Slide)

10 This is one of my last slides to give you an
11 example for the usage of the collagen binding assay. Here
12 we investigated the storage stability of recombinant von
13 Willebrand factor at the concentration of 40 units,
14 ristocetin cofactor units/ml at room temperature. We saw
15 that both von Willebrand antigen, the green line, and the
16 ristocetin cofactor activity seem to be stable over a
17 prolonged period of time, even longer than 70 hours.

18 But, interestingly, the collagen binding assay
19 revealed increase of activity after 3 or 4 hours which was
20 maintained up to 24 hours and then decreased again. This
21 could indicate that there is some structural change
22 occurring in von Willebrand factor upon storage in solution,
23 which was even shown recently by the Sixma group. They
24 published that heating of von Willebrand factor up to 55

1 degrees centigrade increases the tendency to bind to
2 collagen. So, we think that this assay is really indicative
3 for minor structural changes in the von Willebrand factor
4 molecule, and it is a useful tool for assessing the quality
5 of a von Willebrand factor product.
6 (Slide)
7 I would like to summarize. I have presented for
8 you a collagen binding assay for determination of the
9 functional vWF based on a simple ELISA technology, utilizing
10 a covalent immobilization of pepsin-digested type 3 collagen
11 from human placenta to microtiter plates. The precoated
12 microtiter plates are storage stable. The assay is highly
13 sensitive and highly specific for the determination of von
14 Willebrand factor.
15 (Slide)
16 The collagen binding activity correlates with the
17 multimeric structure. This was also published recently by
18 Dr. Aronson, who did the experiment I showed you. He
19 separated the multimer fractions of von Willebrand factor by
20 ultracentrifugation and then made the collagen binding
21 experiment.
22 Collagen binding activity also correlates with
23 ristocetin cofactor activity. The assay may be used for the
24 classification of von Willebrand's disease and is also

1 applicable for quality control of plasma-derived von
2 Willebrand factor concentrates, as well as for recombinant
3 von Willebrand factor.
4 For those who are interested in this assay, I have
5 brought with me a prototype of this assay, and I also would
6 like to invite you to participate in the field evaluation of
7 this assay. So, you have my address in the handouts which
8 you received this morning and please contact me, and thank
9 you for your attention.
10 DR. MONTGOMERY: Our final speaker in this session
11 is Dr. Heinrich Joist, from the St. Louis University where
12 he is Professor of Medicine. He has had an interest in
13 looking at the shear-induced activities of von Willebrand
14 factor and will share some of that with us today.
15 **Assay of von Willebrand Factor Activity Based on**
16 **Shear Stress-Induced Platelet Adhesion/Aggregation**
17 DR. JOIST: I would like to thank Dr. Weinstein
18 for inviting me to participate in this meeting. Von
19 Willebrand factor, as we all know, mediates the interaction
20 of platelets with the injured vessel wall in a strikingly
21 flow-dependent manner. There is some concern that assays
22 like the ristocetin cofactor assay and also collagen binding
23 assay, since they are performed either in a static system or
24 in systems that have either low flow or very poorly defined

1 flow, might not be suitable for measuring the full
2 biological activity of von Willebrand factor.
3 What I would like to do today is not present you a
4 new assay, but discuss with you some of the concepts of
5 relationship between flow and platelet aggregation and
6 adhesion in relationship to von Willebrand factor, and set
7 the background for the development of a shear-mediated or
8 flow-mediated quantitative assay for von Willebrand factor.
9 (Slide)
10 As you well know, one of the first models that was
11 developed for measuring the effects of flow on platelet
12 interaction with an active surface is the Baumgartner
13 annular perfusion chamber. You can see here that there is a
14 strikingly flow-dependent development of platelet aggregates
15 on the surface. This is a denuded rabbit aorta that has
16 been placed into a perfusion model. You can see here that
17 in a flow-dependent manner at relatively moderate flow, 600
18 inverse seconds, and I will come back to this in a second,
19 you can see a substantial amount of platelets adhering to
20 the surface, to this injured vascular surface, as well as
21 large amounts of aggregates formed.
22 If you increase the shear stress here, and this is
23 by no means the highest biologically relevant shear stress
24 that we can apply, the aggregates seem to diminish and more

1 adhesion is noticed. If you block platelet aggregation with
2 this compound, prostaglandin E1, then you can use this model
3 to look at platelet adhesion selectively.
4 (Slide)
5 We have been using a cone/plate viscometer, and I
6 will quickly explain to those who are not familiar with
7 shear experiments, this is a detailed view of this
8 cone/plate system that is placed into a microscope stand.
9 The cone is driven by motor that is hooked up to a computer
10 and this allows us to preprogram the number of shear
11 stresses, the duration of shear stresses applied to a
12 sample, as well as the total duration and the number of
13 shear stresses. The shear actually takes place in this
14 small chamber between the cone and the plate, static plate.
15 The amount of shear that is exerted on the cell suspension
16 that is placed into this space is essentially dependent on
17 the cone angle, as well as on the velocity and frequency of
18 the rotations.
19 (Slide)
20 This is a view of the actual cone/plate setup.
21 Here is the study plate. Here is the cone. To refresh your
22 memory on some of the terms that we are using in shear
23 experiments, the velocity or the shear rate at the plate is
24 zero and then increases as we go through the diameter of the

1 space between the plate and the cone. At the cone surface
2 it is maximum. This is the diameter of the shear chamber,
3 and the shear rate is essentially determined by dividing the
4 velocity over the diameter of the shear space. Shear
5 stress, in contrast, is the shear rate that we determine by
6 this formula, here, multiplied by the viscosity which is
7 very dependent, of course, in blood on the concentration of
8 red blood cells.
9 (Slide)
10 This is an example of what happens if we resuspend
11 normal platelets in normal plasma, containing a normal
12 concentration of von Willebrand factor, or in plasma from a
13 patient with severe von Willebrand factor type 3. This is
14 platelet aggregation in response to ADP, which is normal as
15 we know. This is in response to ristocetin. There is an
16 absence of aggregation, as we would expect.
17 If we now place these suspensions in the shear
18 model that we use, in the cone/plate, we can see that as we
19 increase the shear stress there is increasing platelet
20 aggregation. This is the control, here, meaning normal von
21 Willebrand factor. And that curve can be shifted towards
22 lower shear stresses by the addition of red blood cells. I
23 will come back to this in a minute.
24 If we have no detectable von Willebrand factor in

1 the plasma, then there is some shear-induced platelet
2 aggregation but it is much reduced, and the difference
3 between von Willebrand disease plasma and normal plasma
4 increases with the shear stress.
5 (Slide)
6 The Rice group has recently developed an
7 epifluorescense video viscometry cone/plate, actually not a
8 cone/plate but a pallo/plate chamber system that is very
9 suitable for measuring quantitatively the amount of platelet
10 deposition on a particular surface. This is the overall
11 system. Blood is directed through this system and then
12 captured here, and can be recirculated if necessary so you
13 can either have a single-pass system or a multiple
14 recirculating system.
15 (Slide)
16 When you look at the data here, this is what
17 happens when you expose blood from a healthy donor for 60
18 seconds to the system. You get a very remarkable
19 demonstration of platelet adhesion and aggregation. If you
20 take blood from a patient with type 3 von Willebrand
21 disease, there is very minimal deposition of platelets on
22 the surface. If you take this blood from a patient with von
23 Willebrand disease type 3 and add purified von Willebrand
24 factor to normalize the amount of von Willebrand factor

1 present, then you can restore most of the ability of
2 platelets to deposit on the surface.
3 It is somewhat difficult to distinguish in this
4 system between platelet adhesion and platelet aggregation.
5 So, what is measured here quantitatively, and Dr. Montgomery
6 showed some data on this, is really total platelet
7 deposition, which is a combination of adhesion and
8 aggregation.
9 (Slide)
10 We have modified our system so that we can not
11 only measure the aggregation that occurs in flow by
12 measuring the loss of single platelets after flow and
13 comparing it to before flow. But we can also insert glass
14 disks, or we are now using plastic disks that are coated
15 with collagen, into the plate of the cone viscometer and
16 then following the shear experiments we can take these disks
17 out and measure the platelet deposition. After appropriate
18 washing, we can determine adhesion apart from aggregation.
19 I will show you a few examples of this.
20 (Slide)
21 This is the effect in this system of plasma von
22 Willebrand factor. This is on adhesion. Here we
23 resuspended normal platelets in normal plasma. You can see
24 here that with increasing shear stress there is increasing

1 adhesion.

2 This is normal platelets in von Willebrand factor-
3 deficient plasma. As you can see here, the difference
4 between normal and vWF-deficient increases as you increase
5 the sheer stress.

6 (Slide)

7 This is an experiment where we tested the possible
8 effects of platelet von Willebrand factor on platelet
9 adhesion. Here, again, are intact platelets with von
10 Willebrand factor-deficient plasma. Here we do the same
11 experiments with NDP degranulated platelets that presumably
12 have no or have very little internal platelet von Willebrand
13 factor. You can see here that there is a marked difference
14 in the adhesion of these platelets in deficient plasma.

15 If you then take degranulated platelets in von
16 Willebrand factor-deficient plasma and add ADP, you actually
17 do not get an increase in the amount of adhesion.

18 (Slide)

19 How do all these experiments fit into a common
20 mechanism of shear-induced platelet aggregation? Some have
21 suggested that what happens with shear is that platelets are
22 directly activated by shear and that the main event that
23 occurs in the activation process is that shear induced an
24 influx of calcium that then modifies, in some way, GP1B to

1 recognize presumably unmodified von Willebrand factor, and
2 also exposes GP2B3A, which then leads not only to platelet
3 adhesion but also to platelet cohesion with von Willebrand
4 factor being the main ligand not only between GP1B but also
5 between GP2B3A on different platelets.
6 (Slide)
7 We have prepared some experiments to test this
8 further. What is shown here is how this experiment was
9 designed to test the role of ADP in shear-induced platelet
10 aggregation. Here, first, you can see what happens when you
11 remove ADP completely by CP/CPK and also block the ADP
12 receptor with ATP. You very quickly come to a situation
13 where there is virtually complete blockage of platelet
14 aggregation in the viscometer.
15 You now take that concentration that completely
16 blocks platelet aggregation and you do a shear experiment,
17 and this is the control experiment with buffer added where
18 you get about 65% platelet aggregation. Here, when you add
19 this mixture that prevents or practically eliminates the
20 role of ADP in platelet aggregation, virtually all platelet
21 aggregation induced by shear is abolished.
22 (Slide)
23 This is showing the important effects of red blood
24 cells on shear-induced platelet aggregation. This is shear-

1 induced platelet aggregation at different hematocrits. You
2 can see that it doesn't really matter much whether you use
3 fresh red blood cells or glutaraldehyde-fixed red blood
4 cells. In fact, particularly in the adhesion experiments it
5 seems that glutaraldehyde-fixed platelets are actually more
6 potent in stimulating and potentiating shear-induced
7 platelet aggregation than normal fresh red blood cells.
8 In other experiments we have shown that the effect
9 of fresh red blood cells on shear-induced platelet
10 aggregation is mediated by both small amounts of ADP being
11 liberated from red blood cells, as well as the physical
12 transport effects of red blood cells that bring the
13 platelets to the boundary surfaces so that they can interact
14 with the surface.
15 (Slide)
16 This shows the effects of red blood cells on
17 shear-induced platelet adhesion in this model that I
18 described, and you can see here that not only is platelet
19 aggregation affected by red blood cells but platelet
20 adhesion is striking potentiated by normal fresh red blood
21 cells, as well as glutaraldehyde-fixed red cells. In fact,
22 glutaraldehyde-fixed red blood cells cannot deform and
23 appear to be much more potent in facilitating platelet
24 adhesion than the fresh red blood cells.

1 (Slide)

2 Again, if you look at the effects of removing ADP
3 from this system, it becomes clear that ADP seems to play a
4 role not only in platelet aggregation induced by shear
5 stress, but also in platelet adhesion in this shear model.

6 (Slide)

7 So, all of these experiments, and experiments done
8 by other groups, suggest that there are some very essential
9 requirements for shear-induced platelet aggregation, and
10 some of them are listed here: ADP is clearly a major
11 determinant of shear-induced platelet aggregation. Calcium
12 is necessary, fibrinogen, intact platelet receptors, 2B3A,
13 as well as GP1B and, finally, von Willebrand factor.

14 (Slide)

15 It is our concept that what happens here likely is
16 that shear-induced platelet aggregation brings platelets to
17 the surface. They can interact with von Willebrand factor
18 altered by the attachment to the surface, the artificial
19 surface, and contact is made. The initial contact then
20 causes activation of platelets, release of small amounts of
21 ADP, and some of it may actually come from red blood cells
22 in the whole blood system, but blood cells also facilitate
23 actually bringing the platelets to the surface and
24 supporting the initial contact between platelets and von

1 Willebrand factor on the surface.

2 ADP then plays a major role in not only the

3 adhesion process but also in the platelet aggregation that

4 ensues after shear-induced platelet adhesion has occurred.

5 We feel that the major determinant of platelet cohesion or

6 aggregation following platelet adhesion is probably -- the

7 major ligand is probably not from von Willebrand factor but

8 fibrinogen.

9 (Slide)

10 How does all that fit into the feasibility of

11 establishing a flow system? Well, what I have listed here

12 is some of the ideal features of a shear-flow test of vWF

13 activity. It should be a simple, robust flow system. It

14 should not be something that is enormously expensive and is

15 only present in a few laboratories, which is unfortunately

16 the case with most of the shear-flow systems that are

17 currently in use. It should be a biologically relevant,

18 standardizable surface. I apologize from colleagues from

19 Austria that I put collagen type 1 on here; it should be

20 collagen type 3 probably. There should be well-defined

21 shear rate stress, and the system should be able to

22 distinguish between low and high shear stress. With high

23 shear stress I am talking about greater than 1500 inverse

24 seconds, which relates to roughly 20 dyn/cm2 i shear stress.

1 It probably should be an anticoagulated whole
2 blood system, and one will have to establish in more detail
3 the most suitable anticoagulant for this. Glutaraldehyde-
4 fixed platelets might be useful here in this system, rather
5 than using normal fresh platelets, because with that you
6 eliminate the platelet von Willebrand factor effects on the
7 results.
8 Standardization should be, obviously, rigorous and
9 there are lots of things that one could discuss here,
10 standard plasma and so forth, standard glutaraldehyde-fixed
11 blood cells perhaps that would lend themselves to much more
12 reproducibility.
13 Finally, the test should be validated, and
14 certainly there should be rigorous comparison with the
15 ristocetin cofactor assay, perhaps the bleeding time, and
16 there needs to be obviously in vivo correlation.
17 Where do we stand? I think there are several
18 models now under development in different laboratories. I
19 am personally very enthusiastic that we will probably come
20 up with a flow system. We have been working on a
21 modification of our cone viscometry system for sometime and
22 have not been very successful in developing this into a
23 quantitative assay. We find that the system, looking at
24 gross platelet aggregation and adhesion, is only sensitive

1 in picking up von Willebrand factor levels when the von
2 Willebrand factor drops below about 40%. That makes this
3 test system, obviously, not very suitable.
4 We think that this is probably due to the fact
5 that we have been using normal platelets in the system.
6 Perhaps using glutaraldehyde-fixed platelets, which might
7 work in this system, might offer a way around this. But so
8 far there are formidable problems with making this into a
9 reproducible, precise and quantitatively adequate assay over
10 the entire range of von Willebrand factor activity. Thank
11 you.
12 DR. MONTGOMERY: Although the time is right for
13 going to lunch, you can compare your quest for knowledge
14 versus your quest for food. We will take a few minutes if
15 there are some questions. We will take about seven minutes
16 at most. The other speakers can also go to the microphone
17 to respond.
18 DR. ARONSON: I just want to comment on something
19 you said, Bob, that you should have a 1:1 activity antigen
20 ratio. There are going to be situations where you can have
21 more. As you make your cryoprecipitate you get rid of all
22 the low molecular weight stuff you started off with, and at
23 that point it probably should be greater than 1:1, and if
24 there is no degradation during preparation it also should be

1 more than 1:1.

2 DR. MONTGOMERY: I think it was important for

3 consistency purposes that whatever that ratio is would be a

4 reasonable measure of consistency. As you looked at those

5 concentrates, at least with von Willebrand plasma, they, in

6 fact, were except that some of them were in excess of a

7 ratio of 1. Trevor?

8 DR. BARROWCLIFFE: Just a comment and a question

9 on your presentation, Bob. First of all in relation to the

10 WHO reference plasma, I do take your point about the

11 difference that you find with pooled normal. I mean, there

12 are two aspects. One is that pooled normal plasma will also

13 differ in different labs. But the other point is that in

14 the last collaborative study that we did on this there was a

15 difference or around, I think, 11% in the overall mean

16 between the value in the standard and the pooled normal

17 plasma. We hope to address that and correct that in the

18 next round of the study which will go on next year. But it

19 is still probably better to have a fixed reference standard,

20 as you said, rather than use pooled normal plasma

21 uncalibrated.

22 DR. MONTGOMERY: Having that standard is crucial

23 for communication.

24 DR. BARROWCLIFFE: Yes. As I said, we hope to

1 improve the situation in the next year. My question really
2 was in relation to the predilution in von Willebrand factor
3 plasma of the concentrates. I probably couldn't quite get
4 it from the slides, but did you actually find that that
5 changed the potency of the concentrates compared to the
6 albumin buffer? Because with Factor VIII you actually get
7 an increase of 25% or even more when you switch to predilute
8 in deficient plasma. Did you find the same thing with the
9 concentrates?
10 DR. MONTGOMERY: I compared three slides side by
11 side, but as you move from buffer to albumin to von
12 Willebrand plasma the activity actually increased, the
13 ristocetin cofactor activity increased.
14 DR. BARROWCLIFFE: So you did get an increase.
15 DR. FEDERICI: One comment and one question. The
16 comment is I certainly suggest to use vWF-depleted or severe
17 vWD patient plasma to do studies of purified von Willebrand
18 factor. This comes from my old experience in Ted
19 Zimmerman's and also my place when we are dealing with
20 purified stuff. When we want to make good correlations with
21 von Willebrand factor purified, I usually purify von
22 Willebrand factor with concentrate in my lab. I also make
23 measurements of ristocetin or antigen by using plasma of
24 severe type 3 vWD or plasma depleted first.

1 DR. MONTGOMERY: At some point some companies
2 which were making immunodepleted Factor VIII plasma were
3 doing it with antibodies to von Willebrand factor. Do you
4 know, is there a commercial source of immunodepleted von
5 Willebrand factor?
6 DR. FEDERICI: Maybe this is another question. If
7 this kind of plasma depleted is available commercially, as
8 far as I know, in Europe there aren't any sources.
9 The second is a question to Dr. Joist. You know,
10 in several collaborations with groups which are dealing with
11 shear stress, we have repeated the experience that the
12 platelet von Willebrand factor is an important issue. Did
13 you perform the same cross experiments by using severe type
14 3 vWD in your cone viscometer to see whether or not there
15 were differences? Because this can be an important issue.
16 DR. JOIST: You mean the concentrate studies?
17 DR. FEDERICI: No, no, I am talking about what you
18 have been doing in your stress by mixing reconstituted blood
19 by different sources, and I mean normal platelets versus
20 plasma-deficient. Did you do the opposite?
21 DR. JOIST: Right, I showed you one slide where we
22 switched, where we resuspended degranulated platelets,
23 without von Willebrand factor, deficient platelets in von
24 Willebrand factor-deficient plasma. And they don't do

1 anything. There is virtually zero shear. Well, it is never
2 zero. There is some adhesion and some aggregation but it is
3 markedly depressed.
4 DR. FEDERICI: Thanks.
5 DR. MAZURIER: I have a comment about my concern
6 about using a very sensitive method to measure the von
7 Willebrand factor content in concentrate. For example, the
8 CBA on the von Willebrand factor antigen assays there is a
9 detection limit around 1 milliunit/ml, and we use such
10 assays to make the potency of concentrates which contain
11 about 50-100 U/ml. So we have large errors due to
12 dilutions. When you compare after ristocetin cofactor
13 activity, which is not very sensitive, to von Willebrand
14 factor antigen or CBA assay, you have a ratio which is not a
15 good ratio because you compare two different methods with
16 two different sensitivities and with different errors.
17 DR. MONTGOMERY: And another question to deal with
18 is you commented that collagen binding correlated with
19 ristocetin, except you obviously showed that when you put a
20 sample at room temperature correlation goes off by a factor
21 of two. I don't know that one would want an assay that has
22 that type of an in vitro -- I mean, I think it has to be
23 standardized to avoid that type of a problem. Does it
24 occur, for instance, with purified von Willebrand factor as

1 opposed to recombinant von Willebrand factor?

2 DR. TURECEK: This does also occur with purified

3 plasma-derived von Willebrand factor, and it is due to

4 certain conditions of storage. It should only give you an

5 example that this is really an indicative assay for

6 assessing structural changes in the von Willebrand factor

7 molecule which cannot be measured with ristocetin cofactor

8 antigen assays.

9 To the other comment before, I disagree in the way

10 that I have shown you that we have linear calibration curves

11 between 10 milliunits to almost 5 units. It depends in

12 which range you want to measure. If you want to measure a

13 concentrate that has a potency of, let's say, 50 units or

14 100 U/ml you have to dilute it 1:10 or 1:100 to maximum.

15 You do not have to go to the very low end of the calibration

16 curves. It really depends on what you want to assay. If

17 you want to assay minor amounts of von Willebrand factor in

18 type 3 or potentially type 1, then you will go down as low

19 as possible.

20 DR. MONTGOMERY: It appears that we have reached

21 the time of our pangs for lunch exceeding our pangs for

22 questions. So, we will reconvene at 1:10.

23 (Whereupon, at 12:20 p.m., the Workshop adjourned

24 for lunch, to reconvene at 1:15 p.m.)

1 AFTERNOON SESSION

2 DR. WHITE: My name is Gil White. I am from the
3 University of North Carolina at Chapel Hill, and this is
4 Session V, which is entitled "Manufacturer's Perspective:
5 Clinical Trials and Pharmacokinetics," and the format will
6 be the same as this morning. We will have six speakers and
7 then field questions to all of those speakers during a
8 discussion period after all six talks.

9 The first talk will be entitled, "Composition,
10 Safety and Efficacy of Humate-P in von Willebrand's
11 Disease," and will be given jointly by Dr. Jorg Friedebold
12 and Alena Dobrkovska, from Centeon Pharma, in Marburg
13 Germany. The first third of the talk will be delivered by
14 Dr. Friedebold and then the second two-thirds of the talk
15 will be by Dr. Dobrkovska. Dr. Friedebold?

16 **Manufacturer's Perspective: Clinical Trials;**
17 **Pharmacokinetics**
18 **Composition, Safety and Efficacy of Humate-P**
19 **in von Willebrand's Disease**

20 DR. FRIEDEBOLD: Mr. Chairman, ladies and
21 gentlemen, I would like to thank the organizers of this
22 meeting, CBER, for the invitation. We are pleased to have
23 the opportunity to contribute to this topic.

1 (Slide)

2 In the United States Humate-P is widely used for
3 the treatment of von Willebrand disease even though it is
4 not licensed for this indication, as all of you may know.
5 Ninety-five percent of the product sold in the U.S. is used
6 for the treatment of von Willebrand disease.

7 (Slide)

8 Centeon will present laboratory evidence that
9 Humate-P consistently contains a relevant fraction of high
10 molecular weight von Willebrand factor multimers. That will
11 be done by myself. After that, preliminary pharmacokinetic
12 data of Humate-P in von Willebrand disease patients will be
13 presented by Dr. Dobrkovska and, third, a summary of
14 experience in von Willebrand patients from Canada who
15 received Humate-P in an emergency release program will be
16 presented.

17 (Slide)

18 This overhead shows analytical data that confirms
19 the consistency of the manufacturing process of Humate-P,
20 and that the product is enriched 2.54-fold in von Willebrand
21 factor activity, plasma 0.26, measured with 47 batches, and
22 the specific activity of von Willebrand factor given as the
23 ratio of ristocetin cofactor to von Willebrand factor
24 antigen content measured by Laurell activity is 0.96

1 plus/minus 0.14 with the same 47 batches, which is close to
2 the value expected for normal human plasma as being 1.
3 (Slide)
4 Here we see an immunostained Western blot of 18
5 different lots of Humate-P compared to normal human plasma,
6 which is in lanes 1, 8, 15 and 22 as a standard.
7 The electrophoresis is carried out on a low
8 concentration agarose gel in the presence of SDS. Lane 3
9 shows a non-representative result due to a sample loading
10 error. This has been reanalyzed on another gel and showed a
11 result comparable to the others. What this shows is an
12 overall high consistency of the multimeric band pattern in
13 the Humate-P lots tested if you compare this level, here.
14 (Slide)
15 Let's turn to the evaluation of the multimeric
16 electrophoresis now. In 1989, Tatiwaki and Takahashi
17 suggested an arbitrary grouping of von Willebrand factor
18 multimers in gels, comprising three different groups: the
19 small multimers, which are on the right side of this
20 picture; the medium or intermediate sized high molecular
21 weight multimers and the real large high molecular weight
22 multimers over here.
23 Nevertheless, it is generally accepted by the
24 community, and published by many authors, for example by

1 Scott and Montgomery, in 1993, that the clinical efficacy of
2 von Willebrand factor is related to the high molecular
3 weight multimers. Moreover, this grouping, 1, 2, 3, enables
4 a reliable comparison of different Factor VIII/von
5 Willebrand factor preparations. So, this was for normal
6 human plasma in this picture.
7 (Slide)
8 This overhead shows the distribution of von
9 Willebrand factor multimers in a representative Humate-P
10 lot. Band 11 in higher, this portion here, comprise a high
11 amount of the total of the von Willebrand factor protein in
12 that lane. This particular batch has a content of 85% high
13 molecular weight multimers compared to band 11 and higher of
14 normal human plasma.
15 (Slide)
16 This slide displays the specific activities of
17 different Factor VIII products from different manufacturers,
18 shown as A, B, C and so on, expressed as the ratio of
19 ristocetin cofactor to von Willebrand factor antigen
20 content. Humate-P here is found at 0.9 approximately, which
21 almost reaches the expected value of normal human plasma of
22 1.
23 (Slide)
24 This graph shows a good correlation between the

1 specific von Willebrand factor activity on the X axis
2 expressed, again, as ristocetin cofactor activity to von
3 Willebrand factor antigen content, to the high molecular
4 weight content expressed in percent relative to normal human
5 plasma, on the Y axis, again, for different Factor VIII
6 concentrates. Humate-P is located in here, in the upper
7 right part.
8 (Slide)
9 So, we can see now an overall high consistency of
10 Humate-P batches, which also implies a high consistency of
11 the manufacturing process by looking again at these numbers,
12 with small deviations and content of about 84.2% plasma and
13 6.3% of high molecular weight multimers.
14 (Slide)
15 As a conclusion, appropriate analytical methods
16 exist which enable the comprehensive and effective
17 characterization of Factor VIII/von Willebrand factor
18 products, including the von Willebrand factor multimer
19 content and size distribution.
20 Using these methods, consistently high product
21 quality has been confirmed for Humate-P. Humate-P
22 reproducibly exhibits a high von Willebrand factor
23 ristocetin cofactor to von Willebrand factor antigen ratio,
24 so there is high specific activity, and contains a large

1 amount of biologically active high molecular weight von
2 Willebrand factor multimers. Thus, regarding its analytical
3 characteristics, Humate-P fulfills all requirements for
4 Factor VIII/von Willebrand factor concentrate highly
5 suitable for the effective treatment of von Willebrand
6 disease.
7 Now I would like to hand over the microphone to
8 Dr. Dobrkovska who will present the clinical data. Thank
9 you.
10 DR. DOBRKOVSKA: Ladies and gentlemen, Mr.
11 Chairman, excuse my voice, I have a little bit of a cold. I
12 would now like to continue with the clinical part.
13 (Slide)
14 I would like to present a pharmacokinetic study
15 interim results on six patients with von Willebrand disease,
16 various types, of Dr. Chediak who, unfortunately, is not
17 quite well and cannot be with us today.
18 There were six von Willebrand patients, two of
19 them with severe type 1, two with type 2A, and two with type
20 3, who received a single dose of Humate which was equivalent
21 to approximately 80 IU of von Willebrand factor/ristocetin
22 cofactor/kilogram body weight. The sampling was done before
23 the infusion and then over the following 50 hours.
24 (Slide)

1 The *in vivo* recovery was calculated in two ways,
2 once as the traditional percent of normal and the other with
3 a way which is expressed as increase in unit/deciliter
4 plasma per doses per kilogram body weight, and not
5 correlated with plasma. So the extensive clinical
6 experience which you have heard about before from various
7 speakers gave empiric knowledge that after each unit of
8 Factor VIII per kilogram body weight you can expect an
9 approximate increase by 2, and by ristocetin factor by at
10 least 1.5. Given the variation of the method for ristocetin
11 cofactor, one can say that this is fairly within the range
12 expected.
13 Here again you see something which was already
14 mentioned today, that the antigen is lower than the
15 ristocetin cofactor. That is most likely an artifact of
16 comparing two different methods together.
17 (Slide)
18 Median half-life of ristocetin cofactor was
19 approximately 11 hours, which is again in keeping with the
20 general experience, and the antigen half-life appears to be
21 somewhat longer, which may have something to do with perhaps
22 the slow degradation of the high multimers in the course of
23 the time. The distribution of volume by steady state is
24 approximately 60 ml/kg body weight, which shows, as

1 expected, distribution space higher than the plasma volume.
2 The mean residence time is again in the expected levels,
3 again longer for the antigen.
4 (Slide)
5 This is also reflected in this curve which shows
6 the course of the values as the half-life of Factor VIII,
7 and these conditions cannot be calculated. You see that
8 after the infusion of 80 U/kg body weight of ristocetin
9 cofactor there is rapid increase in Factor VIII activity, in
10 ristocetin cofactor and in antigen, and you see that the
11 Factor VIII levels are fairly sustained during the whole
12 observation time, which is certainly due to the Factor VIII
13 synthesis of the patients which contributes and maintains
14 the seemingly long half-life. The antigen and the
15 ristocetin cofactor follow the normal decay curve and you
16 can see, however, that by about 20 hours you still have
17 levels above 50 U/ml.
18 (Slide)
19 This is by way of an example to show a typical
20 patient with type 3 disease. This is the infusion value,
21 and you see that after infusion the multimeric structure is
22 almost, not completely but almost normalized. A little bit
23 is missing here. Then it slowly declines over the time
24 until the 50 hours, but still at 50 hours there are some

1 residua of von Willebrand factor.

2 (Slide)

3 The changes in the levels of Factor VIII and
4 ristocetin cofactor and antigen were followed and reflected
5 changes in bleeding time, transient changes in bleeding
6 time, similarly as already described by other authors.
7 Infusion values are all over 15 minutes. Post-infusion, 3
8 patients were completely normalized, 3 partly normalized.
9 Then you see that already at 6 hours the normalization is
10 slowly disappearing and at 22 hours it is practically gone.
11 As was mentioned before, this probably does not directly
12 reflect efficacy because we observe this very often.

13 (Slide)

14 I would also like to introduce the evaluation of
15 the clinical efficacy and safety in a rather large cohort of
16 Canadian patients with von Willebrand disease. The clinical
17 efficacy was retrospectively evaluated by the treating
18 physicians.

19 (Slide)

20 We have two kinds of patients. We have 97
21 patients whose results were reviewed on site with the proper
22 validation of the results. That was our primary population
23 and we will refer here to that. We had an equally large
24 group of patients where the results were only obtained by

1 remote retrieval methods, like telephone, fax and so on. We
2 will not refer to those here because these data were not
3 validated, but essentially they were the same as in the
4 primary population.
5 You see that the main types of von Willebrand
6 disease were included. There were somewhat less female
7 patients than male patients but they were fairly
8 represented. The scale of the age range was over
9 practically all age groups.
10 (Slide)
11 This is the summary of the clinical efficacy. The
12 clinical efficacy was judged as excellent, good, none or
13 non-assessable. There were 97 patients who had 525
14 different events, either various surgeries, bleedings of
15 spontaneous or traumatic origin, and other events were, for
16 example, delivery of several patients or invasive diagnostic
17 methods. Prophylaxis was long-term prophylaxis between
18 various bleedings. Overall, one can say that a large
19 proportion of events had good to excellent efficacy, about
20 86% to 98% by surgery, and this is modified by the fact that
21 in 8% of the patients clinical efficacy could not be
22 evaluated.
23 (Slide)
24 Overall tolerance was very good. In 97 patients

1 there were no serious side effects. There were only 7 mild
2 or moderate, chiefly, effects related to the usage of the
3 preparation, mostly mildly allergic. There was no evidence
4 of transmission of hepatitis or other viruses by this
5 therapy.
6 (Slide)
7 In conclusion, it is possible to say that there is
8 an adequate increase of Factor VIII, ristocetin cofactor and
9 antigen after intravenous infusion of Humate-P; that the
10 half-lives and recoveries are in the expected ranges; that
11 there is complete or partial correction of bleeding time
12 shortly after the infusion, but transient; that there is
13 nearly complete normalization of plasma multimeric structure
14 which then slowly disappears; and there was excellent
15 clinical efficacy in 86-98% of various types of bleedings,
16 surgical interventions, obstetrics and various invasive
17 diagnostic techniques. There was no evidence of viral
18 transmission, and there was good general tolerance and
19 safety. Thank you.
20 DR. WHITE: Thank you very much, Dr. Friedebold
21 and Dr. Dobrkovska. The next speaker is Dr. Anastassios
22 Retzios, who is a Clinical Project Manager with Alpha
23 Therapeutics, in Los Angeles, and he will present studies
24 with Alphanate. The title of his talk is "A High Purity

1 Factor VIII Concentrate in the Treatment of von Willebrand's
2 Disease. Dr. Retzios?

3 **A High Purity Factor VIII Concentrate in the Treatment**
4 **of von Willebrand Disease**

5 DR. RETZIOS: Thank you, Dr. White. I would also
6 like to thank Dr. Mark Weinstein and the organizers of the
7 meeting for the kind invitation.

8 I hope that in the next 25 or 30 minutes I will be
9 able to give you a comprehensive synopsis of the work that
10 we have done in order to characterize the efficacy and
11 safety of Alphanate in von Willebrand disease.

12 (Slide)

13 Alphanate is a high purity von Willebrand factor
14 concentrate that was originally licensed for use in
15 hemophilia A in the summer of 1974.

16 (Slide)

17 The original Alphanate included a single viricidal
18 step in its purification methodology. The purification was
19 provided by PG precipitation, chromatography through a
20 capillary micorose column and salt precipitation.

21 (Slide)

22 The resulting concentrate has a specific activity
23 of approximately 150 Factor VIII U/mg protein. The usual
24 intermediate purity concentrates are present in low level or

1 non-detectable amounts.

2 The major contaminant, if the word can be used in
3 this context, is von Willebrand factor. Here, Alphanate
4 contains 1 unit of ristocetin cofactor activity for every 2
5 units of Factor VIII. The ratio of ristocetin cofactor
6 activity to von Willebrand factor antigen is approximately
7 0.72.

8 (Slide)

9 Here is a gel that shows the multimeric
10 distribution of von Willebrand factor in a number of
11 Alphanate lots, as well as in 2 commercially available
12 concentrates from other manufacturers.

13 As one can see, there is substantial lot-to-lot
14 variability and lot-to-lot consistency in the multimeric
15 distribution of von Willebrand factor in Alphanate, as well
16 as in other concentrates. The very high weight multimers
17 are significantly reduced, although they are not necessarily
18 always absent, as occasional overloading shows.

19 (Slide)

20 Going on to our clinical data, the ATC93-01 was
21 designed to evaluate the efficacy and safety of Alphanate in
22 von Willebrand's disease. It was a multicenter, open-label,
23 uncontrolled study in two parts.

24 In part I we were interested in determining the

1 lab response to a single infusion of Alphanate. All of our
2 patients received a single infusion of 40 units of
3 ristocetin cofactor per/kilogram body weight. If the
4 response was inadequate, they were infused with a higher
5 dose of 60 units of ristocetin cofactor per/kilogram.
6 In part II of the study Alphanate was infused for
7 the management of bleeding episodes and as prophylaxis
8 during surgery.
9 (Slide)
10 The endpoint of the study in part I was adequate
11 lab response at one hour post-infusion. Here I would like
12 to add that ATC93-01 was influenced substantially by
13 clinical studies that Dr. Manucci conducted in the beginning
14 of the '90s with a number of Factor VIII concentrates in a
15 cohort of type 3 patients. The study also conforms very
16 closely -- actually, it exceeds many provisions of the
17 guidelines for studies in von Willebrand disease that Dr.
18 Manucci authored and published on behalf of the ICH
19 subcommittee for von Willebrand's disease.
20 (Slide)
21 However, the guidelines did not provide particular
22 guidance for endpoints in the efficacy part of the protocol,
23 our part II. So, in meetings with CBER and subsequent
24 correspondence at the end of 1994, we arrived at the

1 following endpoints: In at least 75% of patients there
2 won't be any use of cryoprecipitate or alternate Factor VIII
3 concentrate. The blood loss will not exceed expected normal
4 blood loss, and all postoperative bleeding episodes will be
5 controlled by Alphanate.
6 (Slide)
7 To clarify endpoints in part I, adequate lab
8 response at one hour post-infusion was determined as Factor
9 VIII and ristocetin cofactor levels at 50% of normal or
10 above, and the bleeding time should be at least partially
11 corrected. Here are our bleeding time correction criteria,
12 which are in conformance with the guidelines
13 (Slide)
14 Going on to the enrollment into the study, we
15 enrolled 65 patients, 53 participating in part I of the
16 study, 10 were infused for 55 bleeding episodes in part IIa,
17 and 23 in 40 invasive procedures in part IIb. If the
18 numbers do not appear to add up, it is because patients were
19 allowed to participate in more than one part of the study.
20 I do not have enough time to go into detail on our
21 enrollment criteria, but I would like to mention that all of
22 our patients are DDAVP non-responsive or DDAVP is
23 contraindicated in them. Also, all our patients were devoid
24 of Factor VIII and von Willebrand factor inhibitors.

1 (Slide)

2 Specifically in part I, using the new
3 classification of von Willebrand's disease, it appears that
4 we have enrolled equal numbers of type 2A and type 3
5 patients, 22 of each kind. We have 6 type 1 patients, 3 of
6 which can possibly be regarded as type 3 because they have
7 non-detectable levels of von Willebrand factor and have
8 bleeding times in excess of 30 minutes, although a normal
9 pattern of von Willebrand factor can be seen if their
10 radiogram is exposed for over 2 weeks.

11 (Slide)

12 Going on to the blood types in our part I
13 patients, the distribution of blood types is similar to the
14 population as a whole. In this analysis 4 of the type 1
15 patients are all type O.

16 (Slide)

17 Going on to the correction of bleeding time with
18 the original infusion of 40 units of ristocetin cofactor
19 per/kilogram, one can see that from the 49 patients with
20 abnormally prolonged bleeding times 32 corrected partially
21 or fully at 1 hour post-infusion.

22 (Slide)

23 Of the 17 patients that did not respond or showed
24 a response that could not be graded as partial due to our

1 guidelines, 13 were reinfused. Six of those showed partial
2 response, which shows that there may be a relationship
3 between dose levels and response in bleeding time. However,
4 we do not have enough points to determine the linearity of
5 that response.
6 (Slide)
7 Going on to the bleeding time over the duration of
8 the observation period, as one can see, both type 2A
9 patients and type 3 patients saw substantial improvements in
10 bleeding time at 1 hour post-infusion. The response to
11 bleeding time is more sustained in type 2A patients. In
12 type 3 patients the bleeding time starts reverting to
13 baseline at about 6 hours post-infusion, although there is a
14 cohort of patients that shows a more sustained response.
15 (Slide)
16 This is the ristocetin cofactor levels throughout
17 the observation period. All the patients achieved levels of
18 ristocetin cofactor at 50% or above, although the response
19 has been more varied than the typical response that one sees
20 in Factor VIII infusion in hemophiliacs. The mean levels
21 remain at about 50% for at least 6 hours post-infusion.
22 (Slide)
23 Now for the $64,000 question, what is the
24 relationship between decreasing bleeding time and increasing

1 ristocetin cofactor activity? As this slide shows, there is
2 none. These results are in accordance with results that
3 were achieved by Dr. Manucci in his studies in a cohort of
4 the ten type 3 patients that were published in Blood in
5 1992.
6 (Slide)
7 Going on to the possible influence of blood type
8 in correction, there aren't enough data to obtain
9 statistical significance, but if one subtracts those 4
10 patients with normal bleeding times pre-infusion, one can
11 see that there were more non-responders with type O.
12 (Slide)
13 Going on to the pharmacokinetics of ristocetin
14 cofactor activity, prior to discussing this slide I would
15 like to indicate that, first of all, neither 93-01 nor the
16 guidelines were designed or even anticipated a very rigorous
17 pharmacokinetic study. In the beginning of the '90s,
18 response to concentrates was a great unknown. So, most
19 investigators were very interested in limiting the blood
20 rules in order to diminish discomfort to the patient.
21 However, going on, the terminal elimination half-
22 life is approximately 10 for type 2A and type 3 patients.
23 The difference that one can see here between type 2A and
24 type 3 in terminal half-life and in mean residence time is

1 not statistically significant. However, differences in area
2 under the curve, both in clearance and volume of
3 distribution at the steady state, are statistically
4 significant.
5 (Slide)
6 Going on to von Willebrand factor antigen levels
7 throughout the observation period, no great surprises here.
8 The main difference is due to baseline levels.
9 (Slide)
10 Going on to Factor VIII, quite a sustained
11 response in increase in Factor VIII levels for both types,
12 type 3 and type 2A.
13 (Slide)
14 Going on to the multimeric pattern over the
15 observation period with type 3 patients, we do not
16 particularly see significant differential clearance of very
17 high molecular weight multimers in comparison to the low
18 molecular weight multimers.
19 (Slide)
20 The same profile can be seen in the type 2A
21 patients.
22 (Slide)
23 Going on to adverse events that we observed in
24 part I, we had 9 adverse events in 7 subjects in a total of

1 72 administrations of Alphanate. Eight were mild and
2 included light-headedness, urticaria, headaches and nausea,
3 and 1 was severe, erythema multiforma, as part of an
4 anaphylactic reaction in a teenage subject.
5 (Slide)
6 Going on to treatment of bleeding episodes, we had
7 10 subjects that were infused for 55 bleeding episodes.
8 Three of those were type 1, 3 were type 2A and 4 were type
9 3. Of the 51 investigated bleeding episodes, 37 were GI
10 bleeds. In 40/52 bleeding episodes a single infusion of
11 Alphanate was adequate to achieve hemostasis. The maximum
12 amount of infusions was 5 in 1/52 bleeding episodes, and
13 that was a hemorrhage due to a shoulder injury.
14 (Slide)
15 Going on to part IIb, Alphanate as prophylaxis
16 during surgery, 23 subjects were infused in 40 minor,
17 moderate or major surgeries. Four were type 1, 11 were type
18 2A, 1 was type 2B and 7 were type 3. The most common
19 surgery was dental extraction. Actually, we had 14 dental
20 operations in the protocol, 6 GI prognostic procedures, 3
21 orthopedic operations, 2 biopsies, 2 GI surgeries, 1 total
22 hysterectomy where an unannounced appendectomy was also
23 included, a pelvic tumor removal, a double hernia repair, a
24 very extensive hemorrhoidectomy and 9 other procedures.

1 (Slide)

2 Looking at the bleeding time correction after the
3 presurgery dose of 60 units of ristocetin cofactor/kilogram,
4 13 of our subjects in 16 surgeries corrected fully, 8
5 subjects in 10 surgeries corrected partially, and 7 subjects
6 in 9 surgeries did not correct. The percentage of
7 corrections here is approximately almost the same, about
8 75%, as we saw in part I of the protocol.

9 We had adequate hemostasis in all procedures. No
10 cryoprecipitate or alternate Factor VIII concentrate was
11 used. According to our latest audited data, blood loss did
12 not exceed expected blood loss in 85% of procedures.
13 Postoperative bleeding episodes in the treatment period, we
14 had 2 in 40 cases controlled by Alphanate.

15 (Slide)

16 Here is a table with some details on the
17 operations. I would like you to notice the relatively small
18 number of infusions required to achieve hemostasis. As you
19 can see in this table and the next one, most of our dental
20 extractions or dental operations required no more than 2
21 infusions.

22 (Slide)

23 The hemorrhoidectomy that went to some distance,
24 partially due to complications, required 13 infusions. We

1 had 12 infusions in the distal clavicle resection and 10
2 infusions in the abdominal hysterectomy.
3 (Slide)
4 Going on to some details in a selected number of
5 patients, here is patient 2201, a type 2A patient. He shows
6 a very typical dosing pattern. Patients usually received 2
7 infusions daily for the first 2 postoperative days. Then
8 the infusion frequency decreased to 1 infusion daily for the
9 next 2 or 3 days, and infusion frequency dropped even
10 further to 1 infusion every 2 days until the completion of
11 the procedure.
12 The patient corrected bleeding time fully after
13 the first infusion, and the bleeding time remained in the
14 corrected area while the frequency of the infusions was
15 relatively high, but it tended to go to baseline when the
16 frequency was decreased. Independent of the bleeding time,
17 the patient did not present any postoperative bleeding
18 episodes and made a full recovery.
19 (Slide)
20 The same is true in the patient that had a total
21 hysterectomy and appendectomy. Here, again, we see the same
22 dosing pattern of 2 infusions daily for the first 2-3
23 postoperative days. The infusion frequency decreases to
24 approximately 1 infusion per day in the next 2 or 3 days,

1 and then it decreases even further to 1 infusion every 2
2 days. Again the bleeding time corrects fully and, as the
3 infusion frequency drops, bleeding time tends to revert to
4 baseline. Again, the patient did not show any postoperative
5 bleeding episodes and again the patient made a full
6 recuperation.
7 (Slide)
8 Here is a patient who has a double hernia repair
9 operation. This patient showed total correction of bleeding
10 time throughout the treatment period and, again, shows very
11 much the same dosing pattern.
12 (Slide)
13 Here is a very interesting patient because she
14 never corrected the bleeding time in the cervical scraping.
15 Again, a similar dosing pattern and, although the patient
16 did not correct the bleeding time, she actually had blood
17 loss less than anticipated and did not show any
18 postoperative bleeding episodes and she healed well.
19 (Slide)
20 Going on to adverse events in part IIa, we had a
21 mild itching. In part IIb, subject 009 who underwent the
22 rather extensive hemorrhoidectomy. The patient developed
23 deep venous thrombosis in the right popliteal vein after 12
24 days of treatment, 5 days after the second hemorrhoidectomy.

1 The investigators attributed this to the patient's medical
2 condition and to the long period of immobilization. It
3 should be noted here that the patient went on to have a
4 third hemorrhoidectomy and also a vena cava filter
5 installation, all under Alphanate treatment. Subject 901,
6 the one who underwent total hysterectomy, experienced a
7 number of mild events connected with her medical condition
8 and the operation, not to Alphanate.
9 (Slide)
10 Recently we added a second viricidal treatment to
11 the manufacture of Alphanate. Alphanate is now heat treated
12 at 80 degrees for 72 hours. We were, of course, interested
13 in defining the effects of heat treatment in the von
14 Willebrand factor of Alphanate.
15 (Slide)
16 As one can see here from the ratio of activity to
17 antigen in the solvent detergent and in the solvent
18 detergent heat-treated versions of Alphanate, they have
19 hardly changed. In a study that we have undertaken in
20 association with a special coagulation lab at the University
21 of Miami, we investigated collagen binding in a number of
22 Alphanate lots, heat treated and non-heat treated. We did
23 find that there was no difference in the collagen binding to
24 antigen ratio between the heat treated and non-heat treated

1 Alphanate.

2 (Slide)

3 Furthermore, we investigated the effects of heat
4 treatment on the multimer distribution in von Willebrand
5 factor in Alphanate. A number of Alphanate lots were
6 examined both prior to heat treating and post-heat treatment
7 and, as one can see on low resolution electrophoresis and
8 high resolution electrophoresis, there haven't been any
9 changes.

10 (Slide)

11 We were also interested in determining the effects
12 of heat treatment in the clinical environment. So we
13 attached an addendum to ATC93-01. In doing so, we modified
14 partially part I of the protocol and so now part I is
15 essentially a crossover study between Alphanate and
16 Alphanate heat treated. We will have at least 12 subjects
17 in the study. The dose has been set at 60 units of
18 ristocetin cofactor/kilogram. We will be following
19 ristocetin cofactor, antigen and Factor VIII for up to 48
20 hours post-infusion.

21 (Slide)

22 We have inserted two additional blood rows here in
23 order to define the pharmacokinetic parameters with a high
24 level of precision. There have been no changes in part II,

1 apart from the fact that patients will be treated
2 exclusively with the heat-treated Alphanate.
3 (Slide)
4 The status -- we have infused 14 subjects so far
5 in part I7, have completed a whole series of infusions in
6 part IIa. We have treated 2 subjects for 4 bleeding
7 episodes in part IIb, 5 subjects were infused in 5 surgical
8 procedures.
9 (Slide)
10 Some preliminary results -- I don't know if they
11 are very visible here, this is a type 3 and type 2A patient,
12 heat-treated Alphanate versus solvent detergent only
13 Alphanate. As one can see, there haven't been any dramatic
14 differences in levels achieved of ristocetin cofactor,
15 antigen and Factor VIII. What is noteworthy here is the
16 consistency in response in bleeding time in both these
17 patients in the heat-treated and the non-heat-treated
18 versions of Alphanate.
19 (Slide)
20 Now, on to a story not often told, the inhibitors
21 of von Willebrand factor. We had 2 patients, both type 3,
22 that developed inhibitors in our study. Patient 111
23 developed a low titer inhibitor of approximately 1.2
24 Bethesda units after 3 administrations of Alphanate within a

1 week. The investigator thought that that was possibly an
2 anamnestic response, and titers have progressively declined
3 and the subject is now inhibitor-free.
4 In the addendum part of the protocol, subject 606
5 developed a low titer inhibitor, possibly after infusion in
6 part I with heat-treated Alphanate. In this case, we are
7 certain that this was an anamnestic response as the patient
8 had a history of inhibitors.
9 (Slide)
10 I would like to note here that the inhibitor level
11 has always been relatively low and was easily overwhelmed by
12 further infusions of Alphanate as, indeed, it happened in
13 patient 111. So the patients remain treatable even when
14 they have inhibitors.
15 (Slide)
16 On to conclusions, Alphanate can be safely
17 administered at doses up to 60 units of ristocetin cofactor
18 activity per kilogram. Alphanate infusions resulted in
19 hemostatic levels of ristocetin cofactor activity at 1 hour
20 post-infusion. Alphanate corrected the bleeding time fully
21 or partially in 77% of the subjects at 1 hour post-infusion.
22 As our part II shows, Alphanate appears to provide
23 adequate hemostasis in bleeding episodes and as prophylaxis
24 during surgery even in the absence in bleeding time

1 correction.

2 Biochemical characterization suggests that heat

3 treatment does not affect the function of von Willebrand

4 factor in Alphanate, and clinical evaluation of heat-treated

5 Alphanate is currently ongoing.

6 Thank you for your attention.

7 DR. WHITE: Thank you very much, Dr. Retzios.

8 Again, we still have a little bit of a hum down there, if

9 you can find out where it is coming from. It is better than

10 it was, but it is still here.

11 The next speaker is Dr. Claudine Mazurier, who is

12 Director of Preclinical Development at LFB, which is the

13 French National Laboratory for Fractionation and

14 Biotechnology in Lille, France. Dr. Mazurier will tall on

15 "In Vitro Evaluation of the Hemostatic Value of the LFB

16 Vapor Heat Treated von Willebrand Factor Concentrate." Dr.

17 Mazurier?

18 **In Vitro Evaluation of the Hemostatic Value of the**

19 **LFB-VHP vWF Concentrate**

20 DR. MAZURIER: Thank you, Dr. White.

21 (Slide)

22 About nine years ago the CRTS of Lille developed a

23 plasma-derived product specially intended for the treatment

24 of von Willebrand disease. The S/D treated von Willebrand

1 factor concentrate is still produced in the facility of
2 Lille, now belonging to the Laboratoire Francais du
3 Fractionement et des Biotechnologies LFB. It contains 42-70
4 units of ristocetin cofactor activity per milliliter, but 10
5 less Factor VIII coagulant activity. Its specific activity
6 ranges between 50-200 U/mg proteins.
7 I will summarize the tests we have performed
8 during the preclinical development in order to evaluate the
9 functional integrity of von Willebrand factor. Second, I
10 will describe the routine evaluation of the different
11 batches of the present production. Then Doris Menache and
12 Jenny Goudemand will talk about the pharmacokinetic and the
13 clinical efficiency respectively.
14 (Slide)
15 This is a description of the battery of tests we
16 have performed in 89 different industrial batches for the
17 preclinical development.
18 First, we analyzed the multimeric pattern in using
19 at that time high resolution gel. We also measured the von
20 Willebrand factor capacity to bind to different ligands,
21 soluble human collagen, fixed platelets in the presence of
22 ristocetin, fresh-washed platelets in the presence of
23 thrombin and Factor VIII. We also measured the ability of
24 von Willebrand factor to promote platelet adhesion in using

1 the rectangular perfusion chamber system described by
2 Sakariassen.
3 (Slide)
4 This slide exemplifies the data obtained on
5 different industrial batches, presented by colored symbols,
6 compared either to normal plasma, on the left side, or to
7 purified von Willebrand factor obtained on the laboratory
8 scale, on the right side. You see that the von Willebrand
9 factor molecules in the final product are able to
10 specifically bind to collagen, platelet GBIb, platelet GP
11 IIb/IIIa complex and Factor VIII.
12 (Slide)
13 Using the rectangular perfusion system of
14 Sakariassen with reconstituted blood containing indium-
15 labeled platelets, and using a flow rate of 1200 inverse
16 seconds, we obtained these data, expressed in percentage of
17 adhesion as a function of the amount of von Willebrand
18 factor added in the reconstituted blood.
19 The different therapeutic batches are represented
20 by colored symbols. You can see that these batches induced
21 normal adhesion with 1.0 U/ml or slightly lower adhesion.
22 When 0.5 units are added 100% adhesion is obtained for all
23 the batches tested.
24 (Slide)

1 As far as routine quality control of the LFB von
2 Willebrand factor concentrate is concerned, it includes the
3 structural analysis of von Willebrand factor in using low
4 resolution gel. We stained the multimers directly in the
5 gel without transfer, using phosphatase-conjugated
6 polyclonal antibodies and we quantified the high molecular
7 weight multimers up to the 15th, the 10th, the 5th mers in
8 using scanning.
9 (Slide)
10 These are examples of the multimers obtained for
11 the concentrate as compared to the pool of normal plasma
12 analyzed in the same gel. First, my eyes cannot see any
13 significant difference between the concentrate and the
14 normal plasma. Consequently, we have to scan the gel and to
15 measure the percentage of the different multimers. Second,
16 in spite of the standardized electrophoretic conditions,
17 sometimes we can clearly see the triplet structure. In
18 other cases we don't see this triplet structure. The length
19 of migration is sometimes shorter, and we can't quantify
20 accurately the multimers up to the 15th.
21 (Slide)
22 We have validated the quantification of the
23 multimers by analyzing in 7 successive experiments aliquots
24 in given batches of our production. You see that by intra-

1 and inter-assay there is very good evaluation for all the
2 multimers up to the 15th, the 10th and the 5th, with CD
3 lower than 10%.
4 We have also evaluated the robustness of this
5 quantitative evaluation by analyzing the data obtained on
6 the different pools of normal plasma that we store at less
7 than 6 months at -80 degrees Celsius. For example, during
8 the past year we have used 2 pools of normal plasma and you
9 see that the quantitative evaluation of the different
10 multimers is reproducible with CD lower than 5%.
11 Consequently, we may express the percentage of multimers of
12 a given batch as compared to the pool of normal plasma
13 analyzed in the same gel. We express the relative
14 percentage of multimers.
15 (Slide)
16 Using this method of expression, we analyzed the
17 29 last batches of our production in '96 and '97, and found
18 that multimers up to the 10th are 82.5% with a CD around
19 10%. We also measure the ristocetin cofactor activity and
20 put this potency on the label on the product for each batch.
21 The test that we use is a semi-quantitative microscopic
22 assay which has been previously compared to the original
23 aggregometer assay with fresh-washed platelets. During this
24 comparison we found very good correlation coefficient of

1 0.95. We use commercially available platelets fixed with
2 ristocetin. We dilute the sample in albumin and use a
3 plasma standard as the reference. Using this test, we see
4 that our prediction is also consistent, with a mean of 60
5 units of ristocetin cofactor activity per milliliter, and a
6 CV of 10%. For all these batches the specific activity is
7 around 100 U/mg protein.
8 (Slide)
9 The last slide is to compare ristocetin cofactor
10 assay and collagen binding assay for the evaluation of the
11 LFB concentrate. As expected, the CBA assay is far more
12 sensitive than the ristocetin cofactor assay. Its
13 quantification limit is 0.01 U/dl, two times the detection
14 limit. The linearity is very good.
15 As far as accuracy is concerned, the repeatability
16 is better than ristocetin cofactor activity, but we were
17 very disappointed by the reproducibility performed in 7
18 experiments because it is not improved as compared to
19 ristocetin cofactor activity.
20 Nevertheless, we still did the correlation of
21 ristocetin cofactor activity and CBA assay in 44 samples
22 taken during the process of preparation of our concentrate
23 in samples ranging from 0.1 to 100 units of von Willebrand
24 factor antigen per milliliter, and found a good correlation

1 coefficient. Therefore, the CBA assay, as it is easier to
2 standardize and is probably more robust than ristocetin
3 cofactor activity, may be an interesting alternative in the
4 picture.
5 I thank you for your attention, and give the floor
6 to Doris Menache.
7 DR. WHITE: It is a special pleasure to be able to
8 introduce the next speaker, who is a good friend and
9 colleague. Dr. Doris Menache is former Director of Plasma
10 Operations at the American Red Cross, in Arlington, and has
11 now achieved the type of status that all of would like to
12 have achieved, that is the title of consultant. Dr. Menache
13 is going to present the second of three talks on the LFB
14 product, and the title of her talk is "Pharmacokinetics of
15 von Willebrand Factor and Factor VIII Coagulant Activity in
16 Patients with von Willebrand Disease Type 3 and Type 2.

17 **Pharmacokinetics of von Willebrand Factor and Factor VIII**
18 **Coagulant Activity in Patients with von Willebrand Disease**
19 **Type 3 and Type 2**

20 DR. MENACHE: Thank you. I would like to thank
21 Dr. Mark Weinstein for inviting me to this meeting to talk,
22 and to thank Gil White for his very nice introduction, and I
23 wish you to be a very good consultant, as I am.
24 (Slide)

1 Pharmacokinetics of Factor VIII coagulant activity
2 and von Willebrand factor were conducted in patients with
3 von Willebrand disease, using von Willebrand factor human.
4 This product is derived from blood collected by the American
5 Red Cross Blood Services from volunteer donors, and is
6 manufactured by the Laboratoire Francais du Fractionement et
7 des Biotechnologies LFB, Les Ulis, France.
8 Six lots of products were used in the study.
9 Ristocetin cofactor specific activity ranged from 131 to 175
10 U/mg protein. The ratio of ristocetin cofactor to von
11 Willebrand factor antigen ranged from 0.91 to 1.4, as this
12 has already been stated several times. Each lot contained
13 no more than 10 units of Factor VIII for 100 units of
14 ristocetin cofactor activity.
15 (Slide)
16 After obtaining IRB approval and informed consent,
17 9 patients with von Willebrand disease type 3, 6 patients
18 with type 2, 1 with type 2A and 1 patient with type 1/2
19 entered the study. The characteristics of these patients
20 are shown on this slide.
21 (Slide)
22 Patients were administered 1 infusion of von
23 Willebrand factor at a dose of either 50 or 100 units
24 ristocetin cofactor per kilo body weight. The bleeding time

1 was measured pre-infusion and at 1, 4, 8 and 24 hours post-
2 infusion. Assays for ristocetin cofactor, von Willebrand
3 factor antigen, Factor VIII coagulant activity and multimers
4 were performed at the time indicated on this slide, which is
5 pre-infusion and up to 96 hours post-infusion.
6 I would like to stress that all the assays were
7 performed in one central laboratory, the Blood Center of
8 Southeastern Wisconsin, in Milwaukee.
9 (Slide)
10 The data points for Factor VIII for all patients
11 were fitted to a model with a linear time synthesis using
12 the formula shown on this slide, where K1 represents the
13 synthesis rate or, rather, the rate of appearance in the
14 circulation of Factor VIII expressed in units per deciliter
15 per hour. K2 is the decay rate of Factor VIII. A0 is the
16 baseline of Factor VIII, and A1 the infused Factor VIII.
17 The pre-infusion Factor VIII was assumed to be the baseline
18 and the 15 minutes post-infusion increment was used to
19 correct for the Factor VIII present in the preparation. The
20 data points for the decay of von Willebrand factor, both the
21 antigen and ristocetin, were fitted to the one-compartment
22 model according to the formula shown at the bottom of the
23 slide.
24 (Slide)

1 This model assumes a constant rate of Factor VIII
2 synthesis, and assumes that the circulating level of Factor
3 VIII is independent of the level of von Willebrand factor.
4 The catabolic constant for Factor VIII was calculated using
5 the model and the formula I have shown you, but it was also
6 calculated in a traditional fashion, that is, it was not
7 corrected for synthesis and calculations were made using the
8 data point from 24 hours post-infusion to 96 hours post
9 infusion, and you will see why we chose 24 hours in a
10 minute.
11 (Slide)
12 This figure compares the calculated curves using
13 the formula and the experimental data obtained in one single
14 patient. The curve in red is for ristocetin cofactor. The
15 curve in blue is for the antigen and the curve in yellow is
16 for the Factor VIII. On each of these calculated curves you
17 have the experimental data points obtained in this single
18 patient for the three types of activities.
19 Following the administration of the product there
20 is an immediate increase in ristocetin cofactor and in
21 antigen, with the highest level noted at the first sample
22 tested, approximately 15 minutes post-infusion. This is
23 followed, of course, by a decay. The Factor VIII level
24 decreased progressively and, except for one patient, a peak

1 was noted at 24 hours post-infusion. But please note that
2 we have no samples between 8 hours and 24, and no samples
3 between 24 and 48.
4 As you can see, the experimental data fit
5 extremely well with the calculated points. In addition, the
6 curve for Factor VIII indicates that the Factor VIII
7 persists in the circulation longer than the von Willebrand
8 factor, a fact that has been noted very often in patients
9 with von Willebrand disease after the infusion of AHF
10 products containing von Willebrand factor.
11 (Slide)
12 This slide shows the mean results we have obtained
13 in 10 patients with von Willebrand disease type 3. The mean
14 rate of synthesis of Factor VIII, or if you prefer, the mean
15 rate of appearance in the circulation of Factor VIII was
16 found to be 6.4 U/dl/h, and ranged from 4.4 to 8.8. The
17 half-life of Factor VIII was around 17 hours. However, if
18 one analyzed the Factor VIII decay without correcting for
19 synthesis, using the one-compartment model from 24 hours
20 post-infusion to 96 hours post-infusion, then the half-life,
21 of course, is much longer and is 40 hours, which appears
22 much slower than for the von Willebrand factor. The half-
23 life of ristocetin and of von Willebrand factor antigen
24 correspond to what others have found, around 12 hours.

1 Correction of the bleeding time in this population
2 of type 3 patients were strongly dose dependent. The white-
3 filled squares indicated the bleeding times at 1 hour post-
4 infusion and 4 hours post-infusion following the
5 administration of 50 units of ristocetin cofactor per
6 kilogram, while the green-filled squares indicate the
7 bleeding times at 1 hour and 4 hours post-infusion following
8 the administration of 100 units of ristocetin cofactor. At
9 4 hours post-infusion the median bleeding time at a dose of
10 50 units was 9 minutes, whereas the median bleeding time was
11 3 minutes for a dose of 100 U/kg.
12 The results in the type 2 patients indicated a
13 rate of synthesis of 5.5 U/dl/h, with a half-life of 16
14 hours for Factor VIII, 14 or 16 hours for the ristocetin
15 cofactor and for the antigen.
16 Analysis of the pharmacokinetic data indicates and
17 allows us to predict that the repetitive administration of a
18 material containing only von Willebrand factor will result
19 in a continuous rise of Factor VIII up to a plateau, the
20 height of which depends on the Factor VIII rate of release
21 in the circulation.
22 (Slide)
23 As shown on this slide, and this is according to
24 the model, following the administration of 1 dose of 100

1 U/kg ristocetin cofactor and then a daily constant dose of
2 50 U/kg of ristocetin cofactor there should be relatively
3 very small shifts in the Factor VIII levels over time,
4 whereas each infusion would be followed by an immediate
5 increase of ristocetin cofactor followed by a decay.
6 (Slide)
7 These expectations are illustrated in this slide,
8 which shows the data from a patient, type 3, treated with 1
9 dose of 100 units ristocetin cofactor per kilogram 24 hours
10 prior to surgery, and then 50 U/kg every 24 hours for 17
11 days. Pre- and post-infusion data for ristocetin cofactor
12 activity are indicated by the yellow squares. Pre- and
13 post-infusion experimental data for the Factor VIII are
14 indicated in pink. The solid white lines indicate the
15 expected calculated Factor VIII C levels. As you can see,
16 the experimental data fit the expected curve. This regimen
17 resulted in levels ranging from 50 units to 112 units of
18 Factor VIII and ristocetin cofactor between 58 and 200 U/dl.
19 (Slide)
20 I would like to conclude by acknowledging that all
21 this work has been done with the collaboration and together
22 with my colleagues listed above, and I would also like to
23 thank all the clinicians of the cooperative study group who
24 enrolled patients and allowed us to perform this study.

1 Thank you.

2 DR. WHITE: Thank you very much, Doris. The last
3 speaker on the LFB von Willebrand factor concentrate is Dr.
4 Jenny Goudemand, who is from the Hopital Claude Huriez, in
5 Lille, France. The title of her talk is "Clinical
6 Management of Patients with von Willebrand Disease with a
7 Very High Purity vWF Concentrate." Dr. Goudemand?

8 **Clinical Management of Patients with von Willebrand**
9 **Disease with a Very High Purity vWF Concentrate**

10 DR. GOUDEMAND: Thank you very much.

11 (Slide)

12 I will be presenting some data about the French
13 clinical experience with the use of the vWF concentrate
14 manufactured by LFB. Data were collected from three
15 centers, Lille, Lyons and Hopital Cochin in Paris.

16 (Slide)

17 From 1989 to 1997 75 patients have been treated
18 with the vWF concentrate. Most patients, 42, had type 1 von
19 Willebrand disease, with various degrees of severity. The
20 range for bleeding time, Factor VIII and ristocetin cofactor
21 activity are indicated on the columns. Other patients
22 included type 2A, 2B, 2N, type 3 and 7 patients with an
23 acquired von Willebrand factor syndrome.

24 (Slide)

1 Patients unresponsive to DDAVP or with
2 contraindications to that product were treated in 99 various
3 clinical circumstances, either spontaneous bleedings, 15
4 cases, including digestive, genital, mucosal cutaneous
5 bleedings and 1 psoas hematoma occurred in a 2N patient, or
6 minor surgery with 5 days or less of institution in 48
7 cases, mainly dental or gynecologic procedures, or major
8 surgery in 36 cases, mainly orthopedic surgery, 15 cases,
9 including 7 total hip or knee replacements, and 2 of them
10 were undertaken in type 3 patients.
11 (Slide)
12 During these occurrences, 40 lots of vWF
13 concentrate have been used. Each vial is labeled with the
14 ristocetin cofactor, which was 58 plus/minus 13 units per
15 milliliter. This was the only information provided to the
16 users. However, clinicians were aware of the low Factor
17 VIII concentration, less than 10%. Only as a special
18 request, we were informed of the exact Factor VIII
19 concentration of each of these lots. Factor VIII content
20 was specially low, from 0.3 to 3 U/ml in the last 26 lots.
21 (Slide)
22 Patients with type 2N were analyzed together and
23 are not included in the following tables. In case of
24 spontaneous bleedings, patients received a first infusion of

1 47 U/kg ristocetin cofactor, corresponding to 4 U/kg of
2 Factor VIII. This was followed by subsequent infusions,
3 almost the same dosage, every 12 or 24 hours when necessary.
4 This was the case mainly for gastrointestinal bleedings,
5 specially in type 2A. In fact, half of the patients
6 received only 1-3 infusions.
7 You can see the baseline levels and levels
8 measured at 1 hour, 12 hours and 24 hours after infusions.
9 We observed that ristocetin cofactor activity was totally
10 normalized 1 hour after the first infusion. At that time
11 Factor VIII was around 50%. Activity measured at 12 hours
12 showed similar levels of Factor VIII and ristocetin
13 cofactor, around 110% and 120%. After that both activities
14 declined.
15 (Slide)
16 Surgery protocols were established according to
17 the baseline Factor VIII level. If Factor VIII was greater
18 than 20%, 30% in case of major surgery, the patients
19 received only 1 infusion 1 hour prior to surgery. If Factor
20 VIII was equal to or less than 20%, or 30% in case of major
21 surgery, there were two possibilities, either to administer
22 2 infusions prior to surgery, the first one 12 or 24 hours
23 before the procedures, and the second one, 1 hour before
24 surgery. If this was impossible, especially in case of

1 emergency, 1 infusion of Factor VIII was given 15-3- minutes
2 after the infusion of vWF and surgery was started in the
3 following 30 minutes.
4 (Slide)
5 In 31 cases of minor surgery and 23 cases of major
6 surgery patients received only 1 infusion 1 hour prior to
7 surgery. This is the dosage in both situations, 51-55
8 ristocetin factor per kilogram, which represents 5 or 6 U/kg
9 of Factor VIII. You can see the baseline levels of the
10 patients.
11 So, when starting surgery the mean Factor VIII
12 level was 67% or 88% and ristocetin cofactor was around 100%
13 in both groups. Of course, there were no type 3 in this
14 series.
15 (Slide)
16 Eleven procedures were performed following the
17 administration of 2 infusions, around 40 U/kg ristocetin
18 cofactor for both injections in minor surgery, and 50 U/kg
19 for both injections in major surgery. You can also see the
20 baseline levels. At the time of surgery no patient had less
21 than 53% Factor VIII in case of minor surgery, and 69% in
22 case of major surgery. Ristocetin cofactor was totally
23 normalized in the majority of patients.
24 (Slide)

1 In 11 cases patients received von Willebrand
2 factor plus Factor VIII at first infusion. The plasma
3 Factor VIII concentrate was administered at a dosage of
4 around 50 U/kg, while vWF was infused at 50 U/kg. You can
5 see the baseline levels of Factor VIII and ristocetin
6 cofactor. When starting surgery, Factor VIII was greater
7 than 65% in all patients. Ristocetin cofactor was almost
8 normalized, with the exception of one patient with an
9 acquired von Willebrand disease who maintained very low
10 ristocetin cofactor activity while undergoing total hip
11 replacement.
12 (Slide)
13 During the postoperative period vWF was infused at
14 a dosage of 30-35 U/kg ristocetin cofactor activity every 12
15 or 24 hours. This kept the Factor VIII level around 120% or
16 130% and ristocetin cofactor around 80%. Patients received
17 1-11 infusions in the case of minor surgery and 6-16
18 infusions for major surgery.
19 (Slide)
20 I will not detail all the content on this slide on
21 type 2N. In these cases, we mainly have to deal with low
22 Factor VIII levels while ristocetin cofactor might be
23 totally normal. So, in fact, these patients have not been
24 treated differently from the others. For 9 episodes, 2 were

1 treated with 1 infusion prior to surgery; 2, with 2
2 preoperative infusions; and the others, with von Willebrand
3 factor plus Factor VIII.
4 (Slide)
5 We have only limited experience with the vWF
6 binding collagen assay in patients and their replacement
7 therapy. When they were performed, we noticed that this
8 assay gave lower values than ristocetin cofactor, especially
9 in type 2A. But, once more, our experience is very limited.
10 (Slide)
11 So, in conclusion, 75 patients affected with
12 different types of von Willebrand disease have been treated
13 with the vWF concentrate in various circumstances. No
14 hemorrhagic complication was observed in any of the
15 patients. Whatever the protocol used, we observed that
16 ristocetin cofactor was generally greater than 90% and
17 Factor VIII greater than 60% at surgery, mounting to greater
18 than 80% and 110% respectively in the postoperative period.
19 (Slide)
20 When tested, the collagen-binding assay gave
21 slightly lower values than ristocetin cofactor. Ristocetin
22 cofactor was the only information given to the clinicians,
23 aware of the los Factor VIII content.
24 Lastly, ristocetin cofactor allowed modulation not

1 only of the von Willebrand factor but also the Factor VIII
2 concentration, which is specially important in the case of
3 surgery and provided efficient therapeutic protocols.
4 Thank you.
5 DR. WHITE: Thank you very much, Dr. Goudemand.
6 The last speaker of this session is Dr. Hans Peter Schwarz,
7 who is Director of Research and Development in the Division
8 of Blood Products and Therapeutics at Immuno, in Vienna,
9 Austria. The title of his talk is "Preclinical Evaluation
10 of Recombinant von Willebrand Factor."

11 **Preclinical Evaluation of Recombinant von Willebrand Factor**

12 DR. SCHWARZ: Good afternoon, Dr. White. Good
13 afternoon, ladies and gentlemen. First of all, I would like
14 to thank Mark Weinstein for the kind invitation to
15 participate in this meeting, and we are very honored, from
16 Vienna, to be able to be here.
17 Being the last speaker, I have the privilege to
18 think over what we heard in the last few hours, and I think
19 I will have to disappoint you because my presentation will
20 not contribute to any clarification and to the objectives of
21 this meeting, rather, I think it will increase the overall
22 confusion regarding assays and related problems.
23 (Slide)
24 Being the last speaker, I can also show you an

1 introductory slide to von Willebrand factor, and maybe the
2 problems arise from the fact that this protein is too
3 complicated. It has too many functions. And we,
4 clinicians, have to blame the protein for being too
5 complicated.
6 Anyway, this is just a structural outline with the
7 various domains of the von Willebrand factor. There is a
8 large propeptide. There is a Factor VIII binding site.
9 There are binding sites for the glycoprotein 1B, heparin,
10 collagen and other collagen-binding sites, and platelet
11 receptor binding sites.
12 As you are all aware, C terminal dimers form in
13 the ER and end terminal multimerization forms in the Golgi
14 and post-Golgi, and this protein grows to become the largest
15 plasma protein in the circulation.
16 (Slide)
17 Now, von Willebrand factor is synthesized in
18 endothelial cells as a 2791 amino acid containing the pro-
19 von Willebrand factor molecule. Pro-von Willebrand factor
20 consists of, as I mentioned before, a large propolypeptide
21 which contains 741 amino acids, and this is also called von
22 Willebrand antigen-2, and a mature von Willebrand factor
23 monomer.
24 (Slide)

1 We know that about 95% of the von Willebrand
2 factor is secreted via the so-called constitutive pathway,
3 and this consists of incompletely processed material which
4 has a limited degree of polymerization and is functionally
5 immature. Also, some mature von Willebrand factor and the
6 propeptide is released or secreted via this pathway. About
7 5% of the synthesized von Willebrand factor is stored in the
8 Weibel-Palade bodies or in the alpha granules of platelets
9 and this is fully processes; it is biologically active and
10 only released upon stimulation.
11 (Slide)
12 Why is this important for considering recombinant
13 von Willebrand factor? Based on these facts and
14 observations that there is both processed propeptide freed
15 from von Willebrand factor as well as unprocessed material
16 in the human body in the circulation, we made the decision
17 to make both, two candidate preparations of von Willebrand
18 factor, one which is fully processed and another one which
19 is a mixture of processes and unprocessed material.
20 (Slide)
21 So, I will show you some preclinical data of our
22 recombinant von Willebrand factor candidate 1, which is
23 fully processed, and this is achieved by furin coexpression,
24 and furin is a propeptide processing enzyme; it is a

1 heptidase which cleaves arg sequences, and I will show you
2 data on candidate 2, which contains 50% pro-von Willebrand
3 factor unprocessed, having the propeptide linked to the
4 mature form and processed material. Of course, there is no
5 furin coexpression involved in candidate 2.
6 (Slide)
7 This material is expressed in CHO cells.
8 Candidate 1 is purified by affinity chromatography using
9 various steps of heparin sepharose. It is fully
10 glycosylated and multimerized, and in vitro
11 characterizations suggest that it binds to collagen under
12 high shear rate conditions compared to plasma-derived von
13 Willebrand factor and it stabilizes Factor VIII in vivo.
14 (Slide)
15 This is a comparison of the multimers of plasma-
16 derived von Willebrand factor with the recombinant von
17 Willebrand factor. Using a 2% agarose gel, the multimers in
18 plasma dissolve in the classical triplet structure, having
19 the intermediate band and the lower and faster migrating
20 band. This is apparently not the case for the multimers
21 present in the CHO-derived recombinant material.
22 (Slide)
23 This is also shown on this slide using two-
24 dimensional gel electrophoresis to demonstrate differences

1 between plasma-derived von Willebrand factor, von Willebrand
2 factor circulating in plasma and recombinant material. All
3 the multimers in the recombinant von Willebrand factor
4 consist of intact subunits of about 225,000 molecular
5 weight. No other split products are visible.
6 But if you look at the left side of the slide you
7 see the same analysis performed for the plasma-derived von
8 Willebrand factor, and you can see that the intermediate
9 band of the multimer consists of three different structures,
10 one having a molecular weight of 225,000, a band of 140,000
11 and 85,000, which is also demonstrated here. Now, the fast
12 band of the triplet contains two bands, 225,000 and 140,000,
13 and the lower migrating band has 225,000 and another band of
14 molecular 80,000. So thee is a real difference between
15 recombinant and plasma-derived von Willebrand factor
16 (Slide)
17 Is this a concern for us? Does it mean that this
18 is something not physiologic that we have in our hands,
19 derived from CHO cells? This is again a comparison of the
20 multimeric structure between plasma recombinant platelet-
21 derived and endothelial cell-derived von Willebrand factor.
22 You see that, in fact, only the plasma-derived von
23 Willebrand factor multimers consist of the triplet structure
24 with two satellite bands and one intermediate, recombinant

1 only intact multimers. This is also true for platelet-
2 derived von Willebrand factor, as well as endothelial-
3 derived human von Willebrand factor. For platelet and
4 endothelial, no satellite band formation is detectable using
5 these methods.
6 (Slide)
7 Where does the satellite triplet structure derive
8 from? There is apparently a specific protease which cleaves
9 the von Willebrand factor supplement at this tyrosine 842
10 methyrine 843, generating two polypeptides of this molecular
11 weight.
12 (Slide)
13 This is the so-called depolymerase or von
14 Willebrand factor-specific protease, which is a high
15 molecular weight protein which needs to be activated to
16 cleave von Willebrand factor. It is activated by low salt
17 concentrations or urea or conditions of high shear stress.
18 It seems that high shear and all these other influences,
19 such as low ionic strength or guanidine chloride -- they
20 reside into a modulation of the three-dimensional structure
21 of von Willebrand factor, and then this will lead to an
22 exposure of the susceptible binding sites within the subunit
23 of the dimer.
24 This protease does not degrade fibrinogen,

1 collagen or albumin. It is not inhibited by leupeptin or
2 classic serine protease inhibitors. it is also not present
3 in platelets. Recently it was found that the activity of
4 this protease is either absent or defective in chronic
5 relapsing TTP.
6 So, we are currently collaborating with Furlan,
7 from Berne, to address different issues regarding the
8 protease and recombinant von Willebrand factor.
9 You saw this morning that purified protease can
10 cleave recombinant von Willebrand factor in vitro in the
11 presence of high concentrations of urea. However, you will
12 see on the following slide that ex vivo, after the
13 administration of recombinant von Willebrand factor into
14 dogs or pigs with severe von Willebrand disease there is no
15 processing, no proteolytic degradation of the intact
16 multimer.
17 This is obviously an area of great interest.
18 Where does this protease really function in vivo? At which
19 stage is it an artifact during blood drawing, fractionation?
20 Where is the site of the action of this protease in vivo?
21 (Slide)
22 I will show you some data we generated in infusion
23 studies using dogs with severe von Willebrand factor
24 disease. We have a dog colony in Vienna and I will show you

1 data generated with the pigs that we see with von Willebrand
2 disease, which is type 3 in humans. This was in
3 collaboration with the Institut National de la Recherche
4 Agronomique, in France.
5 (Slide)
6 The objectives were, of course, to evaluate the in
7 vivo recovery and half-life of human recombinant von
8 Willebrand factor to see whether or not there is an effect
9 of human recombinant von Willebrand factor on porcine and
10 canine Factor VIII in vivo, and to evaluate if there are any
11 effects of human recombinant von Willebrand factor on
12 primary hemostasis in those animals.
13 (Slide)
14 This is just to demonstrate that the pigs are
15 really deficient in von Willebrand factor antigen. It is
16 below the level of detection. Factor VIII activities are
17 higher than what we know from humans with severe von
18 Willebrand disease. They are between 10% and 24% using the
19 two-stage clotting assay, and similar results using the
20 chromogenic assay.
21 (Slide)
22 This is just a slide to remind the audience that t
23 here are differences in the stoichiometry in the human and
24 the porcine system. You should not forget looking at animal

1 data that the Factor VIII concentration in humans differs
2 very much from this concentration in the porcine system. We
3 only have 0.1 mcg/ml in the human but von Willebrand factor
4 antigen concentration of 10 mcg/ml. So we can assume that
5 1% to 8% of the von Willebrand factor in the circulation
6 will be saturated with Factor VIII, or 1 Factor VIII
7 molecule per 50-100 von Willebrand factor multimers will
8 bind.
9 Now, how is it in the porcine? Porcine has a 10-
10 fold greater Factor VIII activity. This greater activity is
11 presumably due to the fact that there is a lower
12 dissociation rate in the porcine Factor VIII molecule. The
13 A2 domain dissociates more slowly from the activation
14 complex than in the human system, and relatively von
15 Willebrand factor antigen concentration is lower than in
16 humans. So, more than 50% of the von Willebrand factor in a
17 pig is saturated with Factor VIII.
18 (Slide)
19 Primary hemostasis, a surrogate marker of
20 efficacy, was evaluated by the so-called ear-emersion
21 bleeding time where standardized incisions are performed at
22 the edge of the pig's ear and the ear is placed in a beaker,
23 and you can either measure bleeding time or measure the
24 hemoglobin content in this liquid. The pigs bleed more than

1 30 minutes and they would apparently bleed to death, and
2 cessation is achieved by electric cauterization.
3 (Slide)
4 This is a 2% agarose analysis of blood samples
5 taken after the infusion of 35 ristocetin cofactor units in
6 such a pig with severe von Willebrand's disease. This
7 demonstrates the metabolic clearance of the infused
8 multimers over time. You will appreciate that the high
9 molecular forms of the multimers are cleared faster from the
10 circulation than the low molecular weight forms.
11 (Slide)
12 This summarizes one experiment demonstrating
13 results of some biologic assays. the red bars indicate the
14 bleeding time. It was longer than 30 minutes. Cessation
15 was performed by electric cauterization. Also, this was
16 true for the measurement taken at 3 hours after the
17 administration of recombinant von Willebrand factor.
18 However, there was a surprising finding at 24 hours after
19 this single dose administration, there was a spontaneous
20 cessation of bleeding at about 30 minutes, and this effect
21 on primary hemostasis was sustained for another 12 hours
22 because at 32 hours there was still a spontaneous cessation
23 of the prolonged bleeding in this single experiment.
24 We also see that upon administration of

1 recombinant von Willebrand factor there is a rapid rise of
2 endogenous porcine Factor VIII. This is the insert of the
3 metabolic clearance of the multimers in this experiment.
4 You also see von Willebrand factor antigen levels, and the
5 discrepancy between ristocetin cofactor activity and antigen
6 after this infusion experiment.
7 (Slide)
8 This is another experiment using 70 ristocetin
9 cofactor units and the disappearance of multimers over time.
10 For longer than 70 hours recombinant von Willebrand factor
11 multimers are detectable in animals.
12 (Slide)
13 This just shows you the remarkable effect of human
14 recombinant von Willebrand factor on stabilizing porcine
15 Factor VIII. At this time point hardly any multimers are
16 detectable in the circulation. However, Factor VIII is
17 still 2- or 3-fold increased as compared to baseline. No
18 ristocetin cofactor activity is detectable at this time
19 point.
20 (Slide)
21 This summarizes some of the infusion experiments,
22 and shows differences in half-life between human, porcine
23 and recombinant von Willebrand factor in the porcine model
24 of severe von Willebrand disease. The mean half-life for

1 porcine von Willebrand factor in the pigs is about 7 hours.
2 Human plasma-derived, one experiment, 7 hour half-life, and
3 for the recombinant, three experiments were performed, 32
4 hours, 11 hours and 16 hours. There is a trend to suggest
5 that the half-life of the recombinant material is
6 substantially longer than porcine and human.
7 (Slide)
8 This is representative of the Dutch Quaker dogs.
9 These are the dogs with severe von Willebrand's disease. We
10 have recently identified the molecular defect underlying
11 this disease, and this is caused by a splicide mutation
12 resulting in a mutation within the von Willebrand factor
13 propeptide.
14 (Slide)
15 Despite the fact that no von Willebrand factor
16 antigen is detectable in dogs with severe von Willebrand's
17 disease, they have relatively high Factor VIII activity
18 levels in the circulation, a mean of 54% using the two-stage
19 clotting and 52% using the chromogenic assay.
20 (Slide)
21 This is the normal dog, the multimeric composition
22 of a normal dog, and 5 dogs with severe von Willebrand's
23 disease. They have spontaneous mucous bleeding, nose
24 bleeds, GI bleeds, though their symptoms resemble the

1 symptoms known in patients with type 3 von Willebrand's
2 disease.
3 (Slide)
4 Here again, plasma samples were taken and analyzed
5 on a 2% agarose gel after the administration of 35
6 ristocetin cofactor units times zero. The disappearance of
7 the high molecular weight forms of recombinant von
8 Willebrand factor is apparently faster than the lower
9 molecular weight forms. There is no indication of a
10 satellite band formation over time in the animals.
11 (Slide)
12 The red bars again indicate bleeding intensity,
13 which is expressed as the blood loss in microliters per
14 minute out of cuticle wounds. There is some decrease in the
15 bleeding intensity after 3 hours. However, with no
16 experiment was there a cessation of bleeding from cuticle
17 wounds after the administration of recombinant von
18 Willebrand factor. There is a very rapid rise in canine
19 Factor VIII upon administration of the recombinant material,
20 and a very long-lasting effect of stabilizing Factor VIII at
21 times when von Willebrand factor antigen had disappeared
22 from the circulation.
23 (Slide)
24 This is just another experiment using a higher

1 dose, and again a substantial increase in canine Factor
2 VIII. The blue curves indicate the von Willebrand factor
3 antigen measurements, and the green curves the ristocetin
4 cofactor activity.

5 Also, experiments here are limited because of the
6 few number of experiments performed, but also here there is
7 a clear impression that the half-life of the recombinant von
8 Willebrand factor is substantially longer, 21, 22 and 12
9 hours, as compared to one experiment using plasma-derived
10 material. This slide also shows the recovery data.

11 In essence, we need more experiments, of course,
12 and it is clear that the half-life of the recombinant
13 material is longer than the plasma-derived equivalence. The
14 reasons for this currently aren't clear, but it is very
15 likely that this is due to the fact that the recombinant von
16 Willebrand factor represents an intact molecule structure
17 and is not processed. But there might be other reasons for
18 that prolonged half-life.

19 (Slide)

20 Here we had occasion to test a fraction of
21 recombinant material just containing low molecular weight
22 multimers in a canine experiment.

23 (Slide)

24 It just shows that if you administer recombinant

1 von Willebrand factor which lacks intermediate and high
2 molecular forms you also obtain a significant Factor VIII
3 stabilizing effect, and this is only to confirm in vivo what
4 was known for quite a while for in vitro data, that the
5 Factor VIII stabilizing effect is independent of the degree
6 of multimerization.
7 (Slide)
8 This is a dog which had a severe and almost life-
9 threatening nose bleed. He was treated with 75 ristocetin
10 cofactor units of the recombinant material, and cessation of
11 this nose bleed occurred within 3 hours without any
12 concomitant treatments.
13 (Slide)
14 This is just again the multimers of the blood
15 samples taken after this emergency treatment in the dog.
16 The high molecular forms already disappeared within 30
17 minutes after the administration, and at 24 hours only very
18 few multimers are still detectable in the circulation.
19 Despite this interesting observation of rapid clearance of
20 multimers in an animal which has a bleeding problem, there
21 was a clear hemostatic effect.
22 (Slide)
23 I can summarize what we learnt from infusion
24 studies with the recombinant von Willebrand factor candidate

1. Of course, this material was well tolerated in dogs and pigs. No thrombocytopenia occurred, and the biologic activity of recombinant von Willebrand factor was demonstrated by some surrogate markers, bleeding time, effective stabilization, in vivo. One single experiment would suggest that it has clinical efficacy, at least in the canine model.

(Slide)

Let me turn now to candidate 2, which is a mixture of unprocessed and processed von Willebrand factor.

(Slide)

Just to remind you that furin, which we used in candidate 1, would cleave the propeptide at this site.

(Slide)

Candidate 2 has a specific activity of 60 or greater ristocetin cofactor units per milligram protein; a specific antigen content of about 2 mg antigen/mg protein. CHO proteins are below 80 mcg/mg antigen. CHO DNA is below 1 mg/antigen, and mouse IgG below 20 ng/mg antigen. This material is purified using monoclonal antibody affinity chromatography.

(Slide)

Here again is a comparison between candidate 1 and candidate 2 using an SDS-PAGE under reducing conditions.

1 This is the fully processed material and here is a mixture
2 of the processed and unprocessed von Willebrand factor
3 containing the propeptide.
4 (Slide)
5 Here again are SDS-PAGE blood samples taken after
6 the administration of 70 units in a pig with severe von
7 Willebrand's disease. This is the material infused, pro-von
8 Willebrand factor and mature von Willebrand factor. You can
9 see that the pro-von Willebrand factor-containing material
10 disappears somehow faster from the circulation. It is
11 suggested that this is due to propeptide processing which
12 takes place in the circulation.
13 (Slide)
14 Here the same samples were analyzed using 2%
15 agarose gel. If you look at this polymer which is the
16 concentrate which was administered to peak, you will see
17 that this band is a polymer consisting of a mixture of
18 processed and unprocessed material. But within 30 minutes
19 after the infusion you see that this picture changes and
20 really reflects what we saw previously using the processed
21 von Willebrand factor. So this is also suggestive that in
22 vivo in the circulation propeptide removal takes place and
23 these multimers are converted to multimers containing the
24 mature von Willebrand factor. Again, there is no triplet

1 structure formation over time.
2 (Slide)
3 This is an ex vivo experiment which was performed
4 by Ludwig Drouet with pigs. It just shows that 3 hours
5 after the administration of 70 units of this material there
6 is an increase in platelet adhesion to a collagen-containing
7 surface. So, the recombinant material was able to mediate
8 ex vivo platelet adhesion to a nice extent. This was lost
9 72 hours after the infusion.
10 (Slide)
11 This is a similar experiment performed in another
12 dog with severe von Willebrand's disease. Again, the
13 material infused was the mixture of propeptide-containing
14 and processed mature von Willebrand factor. The propeptide-
15 containing material seems to disappear faster, at least on
16 these gels.
17 (Slide)
18 This is a comparison if you do an experiment with
19 plasma-derived von Willebrand factor. In this case it was
20 Humate-P. Of course, Humate-P does not contain unprocessed
21 propeptide-containing von Willebrand factor. So, there is
22 no band here as compared to candidate 2. But please note
23 the difference in the half-life. There is nothing visible
24 at 48 hours in such an animal. If you compare this to our

1 previous slide, at least at 95 hours there was still von
2 Willebrand factor detectable.
3 (Slide)
4 So, again confirmation of prolonged half-life of
5 recombinant material.
6 (Slide)
7 Here again is the analysis of the multimers which
8 in 20 minutes in this case converts to the multimeric
9 pictures consistent with fully processed multimeric
10 composition.
11 (Slide)
12 You can also measure the propeptide release which
13 takes place in the circulation when this material is
14 administered to a dog. Using an ELISA assay, we could see a
15 rapid increase in measurable propeptide and fast elimination
16 of the propeptide from the circulation of the dog. So, this
17 confirms really that propeptide processing of unprocessed
18 von Willebrand factor can take place in the circulation.
19 (Slide)
20 Also, this is not a reason to be worried because
21 Jon Van Mourik and others could demonstrate that there is
22 von Willebrand factor propeptide concentration measurable in
23 normal human plasma; that upon administration of DDAVP
24 propeptide would increase but also unprocessed von

1 Willebrand factor is detectable in normals upon DDAVP
2 administration, but also in situations of inflammation,
3 stress and sepsis shock syndromes. What we really wanted to
4 mimic with the recombinant approach was a physiological
5 situation and I think this is confirmed by the data provided
6 by others.

7 (Slide)

8 An interesting observation we made in one dog was
9 that upon administration of propeptide-containing von
10 Willebrand factor there was a rapid increase in thrombin
11 generation in such an animal. This is very surprising
12 because at that time there is no change in Factor VIII
13 levels whatsoever. We have to keep in mind that only the
14 von Willebrand factor was administered in one animal.
15 However, there is a very rapid increase in thrombin
16 generation.

17 So, there is thrombin generation <u>ex vivo</u> using a
18 platelet-dependent thrombin generation assay, and at
19 different time points the thrombin generated was measured
20 and the decline in this so-called thrombin potential seems
21 to parallel with the propeptide levels in the circulation of
22 the animals. We were also able to confirm this now in other
23 animal studies. So this opens a completely new area of
24 investigation. What is the role of unprocessed von

1 Willebrand factor? What is the role, if any, of propeptide
2 in the circulation? Maybe it will open new development of
3 assays.
4 (Slide)
5 With my last slide just to share our current
6 interest in the propeptide area. I mean, it is well known
7 that the propeptide is very important intracellularly
8 because it mediates the intracellular polymerization of the
9 dimers. There are some extracellular functions already
10 known for the isolated propeptide. It seems to have some
11 tissue factor inducing activity. It binds to laninine and
12 collagen, and also mature von Willebrand factor. This could
13 potentially create a problem. There seems to be competition
14 between the propeptide and mature von Willebrand factor for
15 some binding sites. So, it needs to be investigated whether
16 this could create a problem with the unprocessed material in
17 achieving optimal hemostasis. It has some cytokine
18 activity. The propeptide is also a substrate for
19 transglutaminase and, very interestingly, it was shown that
20 it binds to the very late antigen-4.
21 (Slide)
22 Infusion of candidate 2 was also well tolerated in
23 dogs and pigs. The in vivo properties are comparable to
24 those of candidate 1. I couldn't show you all the details

1 but, in essence, it stabilizes Factor VIII. It prolongs the
2 half-life; it has a prolonged half-life as compared to
3 plasma-derived von Willebrand factor, and it is pretty clear
4 now that propeptide removal takes place in the circulation.
5 (Slide)
6 I would like to acknowledge my co-workers, the
7 Molecular Biology Group, headed by Prof. Dorner, in Vienna,
8 pharmacology, vascular biology and toxicology, and I really
9 would like to acknowledge also the very helpful and fruitful
10 discussions wit Ed Gomperts and Don Baker, and Ludwig
11 Drouet, Jon Mourik and Prof. Mertens and several groups from
12 in France.
13 Thank you very much for your attention.
14 DR. WHITE: Thank you very much, Dr. Schwarz and,
15 again, I apologize to Dr. Retzios and Dr. Schwarz for the
16 feedback that was occurring.
17 Since we have the table up here, it might be
18 easiest to do the questions and answers from the table, so
19 if I could get the speakers to come up and just sit at the
20 table, and then we can perhaps direct questions to the
21 speakers at the table. Dr. Lusher?
22 **Question and Answer Period**
23 DR. LUSHER: These were all fascinating talks,
24 hearing about those studies that have been done with these

1 various products. I have two questions for Dr. Retzios,
2 with the Alphanate studies, the bleeding patients and the
3 surgical patients.
4 You may have indicated this and I missed it, but
5 how was the dose arrived at? For surgery it looked like 60
6 units of ristocetin cofactor per kilogram was given.
7 DR. RETZIOS: Sixty units ristocetin cofactor per
8 kilogram was the initial dose, the presurgery dose. After
9 that, the guidelines for dosing stated that the physicians
10 can dose at 40-60 for the first two to three postoperative
11 days. We recommend reduction of dosing to 20-30 units of
12 ristocetin cofactor activity for the remainder of the
13 treatment period. Dosing did not exactly go according to
14 our guidelines. People reduced the dosing in time rather
15 than units per kilogram. So, infusion frequency changed
16 rather than dosing levels.
17 DR. LUSHER: In terms of the initial dose you
18 chose and the range for thereafter, was that empiric? How
19 did you arrive at that?
20 DR. RETZIOS: How did we arrive at 60 units?
21 Well, first of all, when we first designed the protocol we
22 were certain that a certain number of our investigators were
23 interested in having the bleeding time corrected prior to
24 going into surgery. So, we knew that with using the highest

1 dose that we tested in part I we would have our best
2 opportunity to correct the bleeding time, using 60 units of
3 ristocetin per kilogram. So, we started with that.
4 A number of investigators stated that they
5 wouldn't proceed with surgery unless the bleeding time was
6 at least partially corrected. So, at that point we felt
7 that it was best to set the initial, presurgery dose at 60
8 U/kg.
9 DR. LUSHER: So if you were to, yourself, then
10 write a package insert for this product at this point, I
11 mean as an indication for von Willebrand in surgery, would
12 you then say the initial dose should be 60 ristocetin
13 cofactor units per kilogram?
14 DR. RETZIOS: On the basis of the success of the
15 protocol so far, I don't see why not.
16 DR. LUSHER: Okay. Then I have one more question.
17 In terms of your evaluation of clinical efficacy for the
18 surgical patients, you stated -- and I think it is in the
19 abstract as well -- that blood loss remained below or at
20 levels predicted for normal non-von Willebrand patients
21 subjects. I wondered how you determined that.
22 DR. RETZIOS: How did I determine that?
23 DR. LUSHER: How did you determine what would be
24 normal?

1 DR. RETZIOS: Well, both blood loss prediction and
2 actual blood loss are relatively blunt instruments to use,
3 but they were I think the best under those circumstances.
4 The way that we determined those, we asked the principal
5 investigator to fax to us a prediction of a blood loss for a
6 patient of similar stature, age or sex at least 24 hours
7 prior to the operation. So, we retained that data and then
8 we asked the anesthesiologist or the attending surgeon to
9 estimate the blood loss that occurred during the surgery.
10 In addition, we take CBC prior and 24 hours after
11 the operation. So, we just tried to, you know, really gauge
12 how much blood loss we really had.
13 DR. WHITE: Other questions? Dr. Montgomery?
14 DR. MONTGOMERY: Peter, on the thrombin generation
15 with the propeptide, did you not see that when you infused
16 the material that didn't have the propeptide?
17 DR. SCHWARZ: That is right.
18 DR. MONTGOMERY: As far as the question of whether
19 it is degraded or whether has more rapid clearance, you
20 would expect that if you followed the half-life of the
21 material, if it was clearance it would be related to that
22 fraction that was lost because there would be accelerated
23 clearance of the total material. Was there any difference
24 whether you infused pro-vWF versus vWF? I mean, if you look

1 at your distribution, what do you estimate the amount of
2 pro-vWF is? About 15%, 20%?
3 DR. SCHWARZ: It is more than that. It is about
4 50%. However, on the left-hand side of the gel -- you would
5 believe that it is 50-50. Right? If you analyzed the
6 material. But this did not come out using the samples and
7 applying them to the gels. There is less signal. I don't
8 know the reasons but there is less material visible also
9 when you do measurements of the bands. There is less
10 propeptide-containing von Willebrand factor than there is
11 apparently in the circulation. So, it seems to be a problem
12 of the method, the sensitivity of the SDS-PAGE. Did I
13 answer your question?
14 DR. WHITE: Peter, let me make sure I understand
15 what your answer is. You are saying that on the slide that
16 you showed, on the right-hand side was your concentrate
17 which showed about equal amounts of pro-vWF and mature vWF.
18 Then all of the samples on the left-hand side of that slide
19 were after you had infused the von Willebrand factor, and
20 there the density of the pro-vWF band looked considerably
21 less than the density of the mature vWF.
22 I think what Bob's question is, it was 50% in the
23 concentrate but it looks like it is closer to 10% or 15%
24 once you infuse it. Does that represent a difference in

1 SDS-PAGE, or does it represent a difference in recovery of
2 the two species of von Willebrand factor once you have
3 infused them?
4 DR. SCHWARZ: It could represent a difference in
5 recovery. It could represent that within 20 minutes --
6 because the first sample is either 20 minutes or 30 minutes
7 after the administration -- that within this time you have
8 already removed relatively more propeptide-containing
9 material, or it has been already processed. So, at 20
10 minutes there is a difference between the processed and
11 unprocessed material. The bands are much fainter for the
12 propeptide-containing material.
13 DR. BARROWCLIFFE: I have a couple of questions on
14 the measurement of ristocetin cofactor activity in the
15 products. Maybe Dr. Friedebold and Dr. Mazurier, could you
16 comment on the degree of parallelism with the plasma
17 standard for your products? Secondly, did you look at the
18 effects of prediluting in von Willebrand factor-deficient
19 plasma?
20 DR. MAZURIER: We used the plasma standard because
21 there is no concentrate available. So we predilute the
22 concentrate in albumin because we have previously shown that
23 the predilution in albumin and in plasma-deficient von
24 Willebrand factor, either plasma from a severe type 3

1 patient or immunodepleted plasma, the results are the same.
2 DR. WHITE: Dr. Friedebold, could you comment?
3 DR. MAZURIER: As far as parallelism is concerned,
4 it is difficult to answer because we have a semi-
5 quantitative assay. But it looks proportional.
6 DR. BARROWCLIFFE: Okay, it looks parallel.
7 DR. MAZURIER: Yes, but it is semi-quantitative.
8 DR. FRIEDEBOLD: With our ristocetin cofactor test
9 we also have calibration against the WHO standard, and we
10 predilute in albumin too.
11 DR. BARROWCLIFFE: Did you look at the effects of
12 prediluting in deficient plasma?
13 DR. FRIEDEBOLD: No.
14 DR. BARROWCLIFFE: Could I just have one more
15 question for Peter Schwarz?
16 DR. WHITE: Sure.
17 DR. BARROWCLIFFE: As far as I could tell, the
18 ratio of ristocetin cofactor activity to antigen in your
19 product was quite low, around 0.2 or 0.25. Was that the
20 same for both candidate preparations, and could you comment
21 on why that might be?
22 DR. SCHWARZ: It was pretty much the same. You
23 are absolutely right. I have no further explanations.
24 DR. WHITE: Do you have any thoughts, Trevor?

1 DR. BARROWCLIFFE: No.

2 DR. FEDERICI: I have a question for Dr. Menache.
3 In your presentation, and also in the paper, you point out
4 that the Factor VIII C rate of synthesis is 6 U/dl/h. Did
5 you calculate with a dosage of 100 U/kg, and is there any
6 relationship between the amount of von Willebrand factor
7 that you infuse into the patient? My question is, is it
8 possible to increase this kind of rate if you give more von
9 Willebrand factor, or is it not dependent on the dosage of
10 the concentrate?

11 DR. MENACHE: In the publication, if I recall
12 well, we had 5 patients at 50 units and only 2 patients at
13 100 U/kg. The rate of appearance in the circulation of
14 Factor VIII seems a little bit faster with the higher dose.
15 The mean that you saw here is putting together the 5
16 patients with 50 units and the 100 units. So it was 10
17 infusions. It is a combination of all the results for all
18 the patients because we only have 10.

19 DR. FEDERICI: So what you are saying is that your
20 expectation is that if you give to the patient 200 U/kg you
21 would have a better increase? You don't know?

22 DR. MENACHE: I don't think so.

23 DR. FEDERICI: You don't think so?

24 DR. MENACHE: I don't think so.

1 DR. KESSLER: Perhaps Dr. Goudemand or Dr. Menache
2 mentioned this in their presentations but I don't recall,
3 that is, picking up on Dr. Lusher's idea of what you would
4 suggest if you were going to license your product and have
5 some guidelines for treatment of surgical patients with type
6 3 von Willebrand's disease with the high-purity von
7 Willebrand factor concentrate. Would you being your
8 treatment the night before, 24 hours before surgery in order
9 to assure that your Factor VIII concentration is adequate on
10 the day of surgery?
11 Secondly, you didn't mention whether or not you
12 noted any formation of inhibitors in your patients, and I
13 would like to know if you have any information on that.
14 DR. MENACHE: We have not treated currently many
15 patients. For the patients we have treated, the dosage that
16 we have recommended to evaluate for efficacy is based on the
17 results of the pharmacokinetics. So, they are not pulled
18 out of the air. We recommended for surgery to evaluate
19 treating the patients 24 hours before surgery with 100
20 ristocetin cofactor per kilogram, and then 1 hour before
21 surgery 50 units ristocetin and every day 50 units. The
22 time of treatment will vary depending on the type of surgery
23 and what is expected. So, the physician will have to
24 determine the number of days which, of course, will not be

1 the same if it is a hip surgery or if it is an appendectomy,
2 for example, although we have determined that we need a
3 minimum of treatment days.
4 Now, our experience is limited and we are
5 evaluating the efficacy on the basis of that protocol. We
6 have so far had 1 patient treated for an ankle fusion, and
7 that is the slide that I showed. This patient was treated
8 with 100 units and then 50 units every 24 hours for 10 days,
9 and then 50 units every 48 hours for another 7 days. This
10 same patient had knee prosthesis a year later and the
11 patient was treated the same way.
12 I know that Jenny Goudemand treats differently,
13 and she has much more experience so she should tell you what
14 she does.
15 DR. GOUDEMAND: In this series there were 2
16 patients with type 3 who underwent total knee replacement,
17 and these 2 patients were treated by Yvette Suttan, in
18 Paris, with exactly the same protocol. They received the
19 day before 100 U/kg and the day of surgery 50 U/kg. At the
20 time of surgery they both had Factor VIII levels around 80%.
21 After that they received only 1 infusion per day, 50 U/kg
22 and they kept the Factor VIII level around 100%, 120% maybe.
23 So, I proceed differently but I did not have to treat type 3
24 patients, but usually we gave the first infusion 12 hours

1 before surgery. But maybe it is too early before surgery.
2 DR. WHITE: Jenny or Doris, what would you do in
3 the case of an emergency surgery situation? If it were an
4 automobile accident and you needed immediate hemostasis,
5 what would you do?
6 DR. MENACHE: I would give the first infusion of
7 von Willebrand factor concentrate, one infusion of Factor
8 VIII at 50 U/kg, and then only von Willebrand factor in
9 order to immediately increase the Factor VIII level.
10 DR. WHITE: I would do the same thing.
11 DR. MONTGOMERY: I think it was in the Alpha
12 study, you had 2 patients with antibodies?
13 DR. RETZIOS: Yes.
14 DR. MONTGOMERY: Those were von Willebrand factor
15 antibodies or Factor VIII?
16 DR. RETZIOS: Yes, von Willebrand factor
17 antibodies.
18 DR. MONTGOMERY: Tell me what a Bethesda unit of
19 von Willebrand factor is.
20 DR. RETZIOS: Okay. The study where we determined
21 approximately 1.2 Bethesda units was done by Dr. David
22 Green, at Northwestern --
23 DR. MONTGOMERY: But this is a Bethesda assay
24 against Factor VIII?

1 DR. RETZIOS: No. It is his assay against von
2 Willebrand factor. I think he has published his assay on
3 inhibitors to von Willebrand factor.
4 DR. MONTGOMERY: These are inhibitors of
5 ristocetin cofactor activity.
6 DR. RETZIOS: Well, yes, ristocetin or --
7 DR. MONTGOMERY: I certainly think the likelihood
8 is going to be that the majority of antibodies are not going
9 to be inhibitory there, and it is important that in any
10 clinical studies looking for inhibitors have other methods
11 for that.
12 DR. RETZIOS: David Green has published this
13 assay.
14 DR. WHITE: So one Bethesda unit there is the
15 amount of antibody that neutralizes 50% of the ristocetin
16 cofactor activity in plasma?
17 DR. RETZIOS: Yes.
18 DR. WHITE: It is basically the same assay using
19 ristocetin cofactor as an endpoint.
20 DR. RETZIOS: That is right.
21 DR. FEDERICI: May I just make a comment on this
22 issue of the inhibitors?
23 DR. WHITE: You may, indeed.
24 DR. FEDERICI: We have been following that patient

1 I presented today several times. I don't think it is very
2 good to express the Bethesda units. The assay is a little
3 different. So you have a mixture and you make dilutions and
4 you try to get 50% of inhibition. Okay? But you do an
5 ELISA, for instance, for the residual amount of von
6 Willebrand factor antigen or you can test the ristocetin
7 cofactor in these patients. So, we can roughly calculate
8 this. It is almost the same thing as Bethesda units but as
9 we are dealing with another protein I don't know if it is
10 correct to go through the same definition, but I agree that
11 this is the assay.

12 The question of these two patients is related.
13 Were these patients previously untreated?

14 DR. RETZIOS: No.

15 DR. FEDERICI: So this is interesting --

16 DR. RETZIOS: You know, for the second patient,
17 606, we queried her upon enrollment and we did know that she
18 was infused with cryoprecipitate and other concentrates, and
19 we did know that she had a previous history of inhibitors to
20 von Willebrand factor.

21 DR. FEDERICI: The reason I am asking is not a
22 silly one. You know, all of us should be aware that now we
23 know how to prevent or to know in advance what the chance is
24 for these patients with type 3 to have development of

1 inhibitors. So we have the possibility to test at least if
2 there are wide deletions of the von Willebrand factor gene.
3 By taking DNA, if you go to test deletions, the wider the
4 deletion is the higher the possibility is to get
5 alloantibody for these patients. The patient I presented
6 has the largest deletion I think you can imagine. Thank
7 you.
8 DR. WHITE: I didn't hear. Were either of those
9 patients' molecular genetics known?
10 DR. RETZIOS: No, at least I don't know but I
11 don't think so.
12 DR. JOIST: Joist, St. Louis. Given the
13 possibility at least that super physiological levels of von
14 Willebrand factor might be prothrombotic, I am surprised
15 that none of these trials were designed to infuse the
16 material rather than to give it periodically in boluses.
17 Has anybody experience with infusion of von Willebrand
18 factor preparations?
19 DR. MENACHE: Savage, in the U.K. has done that
20 and has published on continuous infusion with the LFB
21 concentrate, yes.
22 DR. JOIST: Do we know what the expected savings
23 would be if we would infuse it, apart from a safety concern?
24 DR. SCHWARZ: It is widely used in some areas in

1 Germany, continuous infusion of von Willebrand factor
2 concentrates. I think it was published at the ISCH by
3 Aureswald, in Bremen, continuous infusion.
4 DR. FEDERICI: In the issue of 1994, Thrombosis
5 Hemostasis, the group of Hama, Bona, Zimmerman, Carter,
6 Herbert and Rickles published a report about continuous
7 infusion of CO-8 --
8 DR. MENACHE: Yes, but the question was purified
9 concentrate. That is why I answered it.
10 DR. FEDERICI: Yes, but this was just another
11 issue that people were trying to save Factor VIII
12 concentrate to do infusion.
13 DR. WHITE: I wonder if I might ask a question.
14 It sounded like many of you saw and observed that the von
15 Willebrand factor antigen half-life was longer than the von
16 Willebrand factor activity half-life after infusion. I
17 think Dr. Schwarz, in his last slide, showed a nice fall-off
18 on the high molecular weight multimers. I usually think of
19 smaller molecules as being cleared faster than larger
20 molecules. So, I have a series of questions.
21 One is, why are the large molecular weight
22 multimers preferentially cleared? Second, does anybody
23 remember if that same sort of thing was observed with
24 cryoprecipitate, that is, was there a differential clearance

1 between von Willebrand factor activity in antigen? Then,
2 finally, why does the Factor VIII stay up after the von
3 Willebrand factor antigen is long gone?
4 DR. MONTGOMERY: The question on cryo, I can tell
5 you we took a person through a patent ductus with cryo and,
6 very clearly, the high molecular weight multimers are gone
7 earlier. I don't know the answer, whether it is degradation
8 of those to smaller as opposed -- my thoughts have always
9 been that functional multimers get cleared faster because
10 they get used, and that the ones that are left behind are
11 the ones that are not as functional and, therefore, not
12 consumed.
13 DR. SCHWARZ: It could be multiplicity of binding
14 sites that is greater for high molecular forms and then they
15 have a higher potential to have receptor-mediated clearance.
16 But what is really intriguing is the fact that there is no
17 detectable von Willebrand factor antigen, however, Factor
18 VIII is still very high. So, what is the mechanism behind
19 that?
20 DR. WHITE: I think Dr. Mazurier knows the answer
21 to that.
22 DR. MAZURIER: I don't know, but maybe when cryo
23 was infused von Willebrand factor antigen was assayed with
24 the Laurell assay, and we know perfectly that when low

1 molecular weight multimers are analyzed there is an
2 overestimation of von Willebrand factor antigen when using
3 the Laurell assay. So you have a discrepancy between von
4 Willebrand factor antigen and ristocetin cofactor which may
5 be due to overestimation of antigen.
6 DR. WHITE: One possible explanation of the Factor
7 VIII is that if you take a person with severe von
8 Willebrand's and give Factor VIII, of course, you get a
9 short T-1 half of Factor VIII. Maybe there is some
10 saturation of clearance mechanisms by that von Willebrand
11 factor so that it is no longer detectable in the circulation
12 but for some reason it is blocking the clearance of Factor
13 VIII. Is there any evidence for that?
14 DR. RETZIOS: Well, I don't know if there is any
15 evidence on that. First of all, in your first questions
16 regarding differential clearance of multimers, what I have
17 to say is that the data that I have seen here today and the
18 data that I have generated do not particularly show that to
19 be the case. Yes, you may have disappearance of multimers
20 but it is not due to the fact that they disappear
21 preferentially. We have to differentiate between their loss
22 and rate of disappearance. If they are present in much
23 lower amounts to begin with, they will disappear by 12
24 hours, 48 hours if the rate of disappearance is constant.

1 Actually, a lot of the densitometry has been vertical. If
2 you look at the horizontal, as I have sometimes done, you
3 may see that the rate of disappearance actually is the same.
4 The second thing, regarding the stabilization of
5 Factor VIII, although the assay for von Willebrand factor
6 does not detect a lot of von Willebrand factor or detects a
7 minimal amount of it, if you look at the multimers you will
8 see von Willebrand factor there. That may still be
9 stabilizing the Factor VIII. It is probably a case where
10 the assay is not as sensitive at picking up very many
11 amounts of von Willebrand factor.
12 DR. MONTGOMERY: Let me comment on that. We talk
13 about a 50:1 ratio of von Willebrand factor to Factor VIII
14 but, I will bet you, as you come down the multimeric scale
15 that ratio doesn't hold. We know that monomeric von
16 Willebrand factor binds Factor VIII probably not with the
17 same affinity. We know from our dimer defect where only vWF
18 dimers are made that it binds Factor VIII but at reduced
19 levels. But it may well be that a molar level there is
20 still a closer relationship between von Willebrand factor
21 and Factor VIII with the smaller ones.
22 DR. WHITE: All right. Well, with that, I would
23 like to thank the speakers for the session. It has been a
24 fascinating combination of basic science and clinical

1 observations and I would like to give them a hand of
2 applause.
3 (Brief recess)
4 **Panel Discussion and Questions**
5 DR. RICK: I wonder if we should get started. I
6 believe at least one of our panel members has to leave
7 shortly before 5:00. I would like to thank Drs. Federici,
8 Lusher, Montgomery, Pierce and White for participating in
9 the panel discussion. We would like some particularly
10 focused discussion, if we could, on some of the questions
11 that were handed out in your packet and that you see up on
12 the screen, here. There is a lot of information that is
13 asked for, perhaps more than we can expect to get over the
14 next 45 minutes, but at least we would like to touch on the
15 assay question, the in vitro assay reflecting function, in
16 vivo also in terms of some specific questions about the
17 trial designs and how subjects might be selected, and what
18 types of clinical trial designs might be most useful. Then
19 also in terms of dosing, we have discussed that we have been
20 treating with doses that we are uncertain about, whether we
21 need to be at that level or not. Then data collection if
22 there is time.
23 So I would like to open the discussion first with
24 the initial question about the in vitro laboratory

1 measurement if further trials are to be done. Maybe I will
2 ask Bob to start on that, and we would invite all of your
3 participation in this. Please use the microphones.
4 DR. MONTGOMERY: I not sure we can necessarily say
5 for sure what the best assay is. I think we can say that
6 probably there is the most familiarity with ristocetin but
7 it is also probably the one that has a lot more variability
8 laboratory to laboratory.
9 If I can just ask a question, in the Alpha study
10 didn't you attempt to standardize the ristocetin cofactor
11 activity in the individual institutions?
12 DR. RETZIOS: (Not at microphone; inaudible)
13 DR. MONTGOMERY: I thought there was some clinical
14 study in which instruments were actually given to the
15 individual centers.
16 DR. RETZIOS: We had samples sent also to a
17 central lab. In 93-01 and right now also in the addendum of
18 the study, we do send samples to a core lab. So, we have
19 two sets of data, data from the individual sites and data
20 from the core lab.
21 DR. MONTGOMERY: I think when it comes to doing
22 studies on patients it is important that the individual
23 centers do the studies because they need the clinical input
24 but, in actuality, I think because with all of these assays

1 there will be some problems with standardization it is
2 probably preferable that at least some laboratory does them
3 unless it is carefully controlled so that individual
4 laboratories can do it.
5 I think the issue of collagen binding -- I mean,
6 there are problems with both collagen binding and
7 ristocetin, and what you may want for clinical activity may
8 not be what is best, most desired for the reproducibility of
9 manufacture. I have to say that I probably came into this
10 thinking more and more that collagen binding might be more
11 appropriate until I saw Peter's slide that showed what
12 happens when you take purified von Willebrand factor at room
13 temperature.
14 But my feeling is that we probably need a specific
15 activity, meaning an amount of an activity, either
16 ristocetin or collagen binding, over an amount of antigen as
17 being an indicator of sort of reproducibility of
18 manufacture. If we are dealing with a recombinant product,
19 like Immuno's, that ratio may be very different than the
20 Humate-P. It doesn't necessarily mean that there is a
21 correct one or a wrong one, it is that one is reproducible
22 and that when patients are infused it would seem logical to
23 infuse them based upon some type of an activity assay and to
24 monitor that activity after infusion.

1 DR. WEINSTEIN: Just a question on the collagen
2 assay again, if this product became commercially available
3 would you dose on a collagen assay or a ristocetin assay?
4 How would the product be dosed in the case of a recombinant
5 product?
6 DR. SCHWARZ: It depends whether or not in the
7 meantime collagen binding activity will also be addressed by
8 the international standards available. The only standard,
9 as we heard several times today, is the plasma standard
10 which has been calibrated to ristocetin cofactor activity.
11 If this is going to be calibrated for collagen binding
12 activity as well, then collagen binding activity would be an
13 appropriate unitage for clinical applications as well.
14 DR. MONTGOMERY: Does one collagen binding assay
15 give you the same result as another collagen binding assay?
16 In other words, is there comparability between those assays
17 as done, or obviously if everybody is using the same kit it
18 is going to be standardized, which may be your intent. But
19 I think there are other collagen assays that are there, and
20 have these been compared to see whether a unit of collagen
21 binding activity in one assay is the same as another since
22 there may be variability in a ristocetin assay?
23 DR. SCHWARZ: We have addressed this question
24 internally by developing variants of collagen biding assays

1 based on different antibodies directed against von
2 Willebrand factor polyclonals, monoclonals, different
3 collagens.
4 We also tried to follow the described
5 methodologies which, as I have pointed out, involve
6 tremendous amounts of collagen bound to the microtiter
7 plate. If it is done within one laboratory the results are
8 comparable. But we have seen that with the routine collagen
9 assays, as described for ten years, we have difficulties
10 with inter-assay reproducibility and also with intra-assay
11 reproducibility. Coating of the microtiter plates was not
12 really homogeneous although we tried our best to do this
13 properly. This is the reason why we came up with the
14 protocol I presented today with the covalent immobilization
15 of the pepsin-digested type 3 collagen.
16 I hope that there will be a comparison of several
17 collagen-based von Willebrand factor binding assays in the
18 course of the European Pharmacopeia Commission which is
19 currently discussing whether or not collagen should be
20 included as an activity assay for von Willebrand factor
21 containing concentrates. It was restricted for ristocetin
22 cofactor half a year ago and I hope they will add collagen
23 binding as well, but not a special method in general.
24 DR. WHITE: I think this is a hard question to

1 answer. I don't know whether you are asking what is the
2 best in vitro test to ask manufacturers to do to indicate
3 the potency on a bottle. I am not sure whether you are
4 asking what is the best in vitro test to determine whether a
5 patient is responding properly to the concentrate.
6 Those may be two different questions. If the
7 question is the former, I mean, that is a discussable point.
8 If it is the latter, that is, if you are asking what is the
9 best in vitro test to determine whether or not von
10 Willebrand factor is doing what it is supposed to do in a
11 person, I worry a little bit about the tenor of the question
12 because I am not sure there is a best test, and I am not
13 sure that you want to come out of this with a single test.
14 I still think that the combination and battery of
15 tests that we do may be useful in different patients. Some
16 tests may be useful in one patient and will suffice for one
17 patient, but other tests may be necessary in other patients.
18 What happens when you say there is a single test that is the
19 best test is that people stop doing the other tests and they
20 just do the single best test. Then that is the only test
21 you have.
22 DR. LUSHER: In that regard though, Gil, which
23 battery would you suggest? I mean, we have seen the
24 problems and we all recognize the problems with bleeding

1 times. I mean, it is a fairly gross test, depending on who
2 is doing it, and reproducibility leaves something to be
3 desired. It is only transiently corrective. The antigens,
4 as we have heard from many of the clinicians and the surveys
5 we have done, are usually not available to make a clinical
6 decision. So you are left with either Factor VIII or
7 ristocetin cofactor.

8 DR. WHITE: Well, I still wouldn't exclude a
9 bleeding time. I mean, in the middle of the night in most
10 places the bleeding time is the only thing you can do. You
11 are not going to get a Factor VIII and you are not going to
12 get ristocetin cofactor activity. When it is you and a
13 patient bleeding time is the only thing you can do.

14 I don't know, I mean, I am struck by the fact that
15 many patients who seem to do well at surgery do not have any
16 correction of their bleeding time. Does that mean that
17 those patients would have done well if you hadn't given them
18 anything? Does it mean the surgeon was just very careful
19 and tied everything off because they knew it was a person
20 with von Willebrand's disease? That is one possibility.

21 The other possibility is, indeed, that the
22 concentrate did do something and that the bleeding time
23 really doesn't reflect a bleeding tendency. I think all of
24 us have a feeling that maybe bleeding times don't accurately

1 predict platelet function. I think we feel that more in
2 uremic platelet defects -- at least in my case, I feel that
3 more in uremic platelet defects than I do in von
4 Willebrand's disease.
5 If I were going to do clinical studies, I would
6 still want to check all of these things, and at the end of
7 the study I would like to say that the following things seem
8 to correlate with hemostatic effectiveness of the product,
9 bleeding time, collagen-binding activity, ristocetin
10 cofactor activity or whatever correlated. For clinical
11 studies, I would still like to see folks do the whole
12 battery of tests. Maybe I am still stuck in an ancient mode
13 though.
14 DR. LUSHER: If we look at some of the rather
15 extensive observations that have been made, for example by
16 Jenny Goudemand just for one example, where they have looked
17 at all of these things even though they don't get them back
18 for a day or two, yet, have based their clinical judgment
19 and can correlate with the thing that seem to measure the
20 best and correlate with clinical outcome, if I understood
21 correctly, it was the ristocetin cofactor assay.
22 DR. WHITE: No doubt. I mean, you have to make
23 clinical decisions, and you make clinical decisions based on
24 what you have. But retrospective analysis of data can still

1 be useful in terms of saying this is and this is not
2 helpful.
3 DR. FEDERICI: I would like to add this kind of
4 observation, we have to make a distinction. When we want to
5 test a new product we should rely on as many tests as we can
6 get. The other issue is when we want to think about
7 following the efficacy, as far as we know, we don't have all
8 the data available. This comes from the audience today.
9 So, everyone should adjust the dosage by a quick decision by
10 having a test that you can do in about one hour, two hours.
11 So, this is critical. Of course, the test to be used in
12 this kind of assessment should fit this kind of requirement,
13 otherwise you can just make a decision without knowing what
14 is going on.
15 So we have to adjust our goals. I certainly
16 suggest and encourage using as many -- you know, the first
17 generation, the second and maybe the third generation assays
18 to know what is going on in pharmacokinetics when we infuse
19 our patients. Of course, this is a general statement, von
20 Willebrand factor is multifunctional. Why should we rely on
21 only one test? So, we should understand what is going on in
22 the Factor VIII binding assay and in the Factor VIII domain,
23 in the von Willebrand factor collagen domain because we know
24 that there are different epitopes. They are important for

1 function.

2 But this is one story. We have to have as many
3 tests as we can in pharmacokinetics. But, of course, we
4 also have to cope with the fact that by following the
5 patient we should have tests. Bleeding time can be done at
6 the bedside of course. Factor VIII C can be available
7 within one hour. Also, ristocetin cofactor can be done by
8 an aggregometric test in one hour, maybe less.

9 So, can we rely on other assays, like collagen
10 binding? Are we discussing here about doing the collagen-
11 binding assay in an ELISA system only for one patient? It
12 would cost you $1000 I think. So we also have to be
13 realistic about that.

14 So, things are complex but we have to come up with
15 some decisions that really distinguish the situation in
16 trials in terms of pharmacokinetics and efficacy in clinical
17 practice. Of course, this is my opinion.

18 DR. PIERCE: In order to make a correlation
19 between in vitro activity or a battery of in vitro tests and
20 attainment of clinical hemostasis, any time you look to make
21 a correlation you need to have some patients who don't do as
22 well. If you have uniformly positive experience, then you
23 are still in an uncertain zone. So I think having endpoints
24 that offer a broader range than just a dichotomy -- yes, the

1 patient did well or, no, the patient did not do well -- may
2 be helpful in allowing us to better understand the
3 correlation between some of these in vitro tests and
4 clinical outcome.

5 The other point is that if we have two or three in
6 vitro tests which are eventually shown to correlate rather
7 well with clinical outcome and then we evaluated a totally
8 new test, it is not out of the realm of possibility that the
9 new test might actually correlate with the other in vitro
10 tests but then when you try to make the correlation between
11 what happens in the patient clinically in that new test, the
12 correlation might not be as tight.

13 DR. RICK: One last comment and then we should
14 probably move to the second question.

15 DR. TURECEK: Can I make one comment on the
16 assays? I would like to come back to what Dr. Federici
17 said. We have to clearly differentiate whether what we are
18 talking about is clinical assessment of von Willebrand
19 disease or von Willebrand concentrate test.

20 For clinical assessment you should use as many
21 tests as available. For concentrate you should really have
22 a test which is reliable and robust, and this is not the
23 case for ristocetin cofactor assays because, I would remind
24 you, that we depend on a reagent which is very difficult to

1 standardize and these are human platelets. This is not the
2 case for the collagen binding assays and this is the reason
3 why we are in favor of the collagen binding assay.
4 DR. LUSHER: In terms of assaying the ristocetin
5 cofactor content of the concentrate, which I think is
6 probably further down our list here but since this is coming
7 up, presumably all of the manufacturers have assayed their
8 products even though they may not have the ristocetin
9 cofactor value in the package insert or on the label. Do we
10 know what they are using as a standard? Are they all using
11 the WHO plasma standard to assign the ristocetin cofactor
12 potency of the vials, or how is this being done? Do we know
13 that?
14 DR. RICK: Can anyone respond to that?
15 DR. BARROWCLIFFE: Well, Jeanne, I can tell you
16 just about the ones I know about, which is the Haemate P and
17 the 8Y in the U.K. They are both relating to the WHO plasma
18 standard, either directly or indirectly. I am not sure
19 about the Alpha product and the Immuno product but those two
20 certainly are.
21 DR. CHANG: Some of them use the WHO standard and
22 some of them use the WHO standard and generate their own in-
23 house standard.
24 DR. MAZURIER: As far as any LFB product is

1 concerned, we use an in-house standard calibrated against
2 the WHO standard.
3 DR. TURECEK: I can speak for Immuno. What Dr.
4 Chang said applies for us. We calibrate an in-house
5 concentrate standard against the plasma standard. This is
6 then used for routine assaying.
7 DR. BARROWCLIFFE: Can I just put in a word in
8 defense of the much maligned ristocetin cofactor assay?
9 Everybody says it is a really lousy assay and the results
10 are all over the place, but if you look objectively at the
11 data in the collaborative studies, it is really not so bad
12 at all. I mean, in the international collaborative study on
13 the plasma standard that we did some years ago the
14 coefficient of variation between labs was 8%. The Factor
15 VIII clotting assay was 7%. So it is really not so bad, at
16 least on that type of sample.
17 Now, if you look in the concentrate study that
18 Bill Fricke and colleagues did, the variability between labs
19 was around about 15% on most of the concentrates. But the
20 antigen assays had a similar sort of variability. In that
21 situation you have a like versus unlike situation.
22 We have seen from Dr. Mazurier's data that, in
23 fact, you can get very reproducible results with a fairly
24 simple ristocetin cofactor assay and, in her case, the CVs

1 were actually just as good, if not better, with the
2 ristocetin cofactor as the collagen binding. So I think we
3 can probably do better with the ristocetin cofactor assay
4 but I would say it is really not as bad as it is made out to
5 be.

6 DR. RICK; Thank you. I think we should probably
7 move to thinking a little bit about how subjects might be
8 selected for inclusion into clinical trials. Gil, do you
9 have thoughts about that, and Jeanne, perhaps to start off?

10 DR. LUSHER: Well, I guess an easy way to start,
11 and then I will turn it over to Gil, is clearly type 3
12 patients who do not have antibodies to von Willebrand factor
13 and type 2 and type 1 who have low enough levels that they
14 are not candidates for DDAVP I would think would be all good
15 subjects for clinical trials, especially for safety of a
16 product, and then perhaps broken down into subgroups in
17 terms of efficacy studies depending on whether they had
18 severe disease or type 1 disease.

19 DR. WHITE: Yes, I agree with that. I mean, I see
20 no reason to study type 1 patients who are responsive to
21 DDAVP so basically I would say patients who are unresponsive
22 to DDAVP, which includes all type 3's and most type 2's.
23 That would be what I would say.

24 DR. MONTGOMERY: And have been treated multiple

1 times before so that you try to eliminate the new inhibitors
2 at least from the studies that are showing efficacy.
3 DR. WHITE: Again, as with the Factor VIII
4 studies, if antibody formation is going to be a question
5 that is going to be looked at and, of course, it is, then
6 having molecular types on these people is probably going to
7 be important.
8 DR. FEDERICI: Just a comment, I agree with all
9 the definitions. So, I am thinking about what we have
10 written in the protocol of the European Community project.
11 So, what we would like to do in this kind of project which,
12 don't forget, is a three-year project, is to select patients
13 in the first year for this kind of trial, with a crossover
14 between Factor VIII, von Willebrand factor and von
15 Willebrand factor with Factor VIII. So we will do an
16 infusion trial to make sure that there is no response.
17 Of course, I want to comment a little further on
18 the fact that we don't want to use previously untreated
19 patients, of course, and we exclude younger kids. I think
20 that is normal regulation for hemophilia trials. Of course,
21 we are confident that if you have patients who have been
22 given for years, for many years von Willebrand factor
23 concentrate, this situation doesn't have any chance to have
24 inhibitors but, of course, there is a chance to test by

1 genetics right now if we know for sure that 10% of patients
2 with von Willebrand's disease type 3 can develop von
3 Willebrand factor antibody if they have large deletions. So
4 we are confident that by knowing this we should enroll good
5 and appropriate patients. Of course, the issue will also be
6 raised for the type 2.
7 We didn't discuss in enough detail but, you know,
8 it is still an open question whether type 2B can be treated
9 with DDAVP. There are reports of people who are showing
10 that you can give DDAVP to a type 2B, or what kind of type
11 2A or subtype 2A can be treated by DDAVP. So, there is an
12 open question for the type 2B. But, of course, only by
13 discussing with a steering committee in this kind of a
14 multicenter trial will we have the solutions.
15 DR. PIERCE: In deciding which type 1 patients
16 respond sufficient well to desmopressin to make it
17 unreasonable to go into a trial of one of these concentrate
18 products, what specific cut points would people use, and
19 would they use a combination of cut points, such as
20 ristocetin cofactor activity as well as Factor VIII
21 response, or would they just use the former for example?
22 DR. FEDERICI: You know that those type 1 who do
23 not show platelet von Willebrand factor measurable, you have
24 a very prolonged bleeding time, very low amount of von

1 Willebrand factor in their plasma. So if you give DDAVP,
2 usually you have almost low response. Instead of 30 minutes
3 of bleeding time you can end up with 20 minutes and the
4 Factor VIII rises reasonably but not so high and the von
5 Willebrand factor antigen and ristocetin cofactor move from
6 the baseline but is not corrected. I mean, it stays still
7 at low levels, no more than 30, 35. So you don't go for
8 those patients to surgery with DDAVP alone.
9 You know, all these kind of parameters should be
10 discussed in the first steering committee, and this is an
11 important issue because all the partners should agree about
12 these kind of parameters of course. But I think we will
13 come up with a decision and we will make the decision on who
14 the responders are and who are not the responders.
15 DR. PIERCE: In evaluating that type 1 response,
16 do you advocate using bleeding time?
17 DR. FEDERICI: Yes, sure.
18 DR. PIERCE: In conjunction with those other
19 tests.
20 DR. RICK: Any other comments regarding selection
21 of patients?
22 DR. RETZIOS: Actually, when the Alpha study
23 started we were officially requested by CBER to use as
24 enrollment criteria the criteria that were included in the

1 guide that Dr. Manucci offered in the studies for von
2 Willebrand's disease. In that guide he mentions that
3 patients should be enrolled that are DDAVP unresponsive or
4 DDAVP is contraindicated in them.
5 The point is we later went and clarified what
6 unresponsiveness is. Since our endpoints in the study are
7 that the patients should be reaching at least 50% Factor
8 VIII, ristocetin cofactor and have at least a partial
9 correction in their bleeding time, it appears to me that
10 unresponsiveness to DDAVP should meet similar criteria. If
11 the patients do not achieve 50% Factor VIII, ristocetin
12 cofactor or do not show any improvement in their bleeding
13 time, if any of those do not occur the patient should be
14 regarded as DDAVP unresponsive or as having a limited
15 response to DDAVP.
16 DR. MENACHE: Could Dr. Federici provide me with
17 some clarification? The selection of your patients that you
18 are discussing now is for a pharmacokinetic study?
19 DR. FEDERICI: Yes.
20 DR. MENACHE: Not for efficacy?
21 DR. FEDERICI: Not for efficacy. I am sorry, I
22 don't want to go into detail because --
23 DR. MENACHE: No, I understand. When you say you
24 will not include children, this means what age, and if they

1 are type 3 --

2 DR. FEDERICI: Twelve years old.

3 DR. MENACHE: And if these patients are type 3 and

4 they are already under replacement therapy you will not

5 enroll them?

6 DR. FEDERICI: I think this is maybe an open

7 issue, but in the original protocol submitted to the

8 European Community, as far as I remember, it is 12 years

9 old. But, of course, this can be modified and the steering

10 committee could change its position in terms of previously

11 untreated or previously treated.

12 DR. MONTGOMERY: Is your concern that they are

13 going to develop inhibitors, or is your concern that the

14 clearance in a child is different?

15 DR. FEDERICI: We are more concerned about the

16 testing. You know, you are a pediatrician. You know how

17 difficult it is to do bleeding time in those patients. It

18 is one of the parameters we want to test in pharmacokinetics

19 and the bleeding time is still very unpleasant for the

20 patients. So when I see pediatric patients I have problems

21 in convincing the patient, of course, first and then the

22 parents to make at least more than one bleeding times. I am

23 afraid if we ask these kind of patients to repeat bleeding

24 time at least four times, as in the protocol, we will have a

1 lot of troubles. So I have the feeling I don't have enough
2 experience with patients younger than ten years, and for
3 those patients it is also difficult to standardize the
4 bleeding time. Maybe you have better --
5 DR. MONTGOMERY: I don't. I don't feel there is a
6 problem with the bleeding time being different in a child
7 over four or five, other than being irritated by having it
8 done.
9 DR. FEDERICI: Yes, yes, maybe that would be a
10 bias.
11 DR. MACIK: Actually, the question I have as far
12 as patient selection also goes somewhat into the next
13 question of what you are going to study because, although I
14 use DDAVP up front, there are many type 1's who have come in
15 bleeding then require surgery a day or two into it, and you
16 only get so much mileage out of your DDAVP in your type 1's
17 if you have big surgery or you need hemostasis for more than
18 one or two days. So, I would hate to see the type 1's
19 closed out of some of these studies when they are in a
20 situation where DDAVP might not carry them all the way
21 through their planned procedure, or whatever they came in
22 with. Dental surgeries, planned surgeries that are open and
23 shut, those DDAVP covers very well in most patients but we
24 all know there are other situations where you will use up

1 your DDAVP and I would like to be able to include those
2 patients.
3 DR. FEDERICI: So, if I understood exactly, your
4 comment is related to long treating with DDAVP. Am I right?
5 DR. MACIK: Right.
6 DR. FEDERICI: If you have a patient with type 1
7 and DDAVP and you go through an operation that takes --
8 usually we try to cope with this problem, you know, the
9 problem of tachyphylaxis by giving DDAVP three, four times
10 in a certain period of time. Then, if we are lucky -- it
11 depend son the operation, of course, and the surgery. If
12 you can give at least four shots of DDAVP you can survive 48
13 hours. Then, hopefully, if the patient doesn't have any
14 bleeding you can stop your infusion for maybe one or two
15 days, waiting for the new synthesis of von Willebrand factor
16 in endothelial cells. Then you start over --
17 DR. MACIK: Right, which sometimes you have the
18 ability to do but I know that depending how low your von
19 Willebrand is. I mean, if you have a mild von Willebrand
20 you can usually get away with that. If you have someone who
21 has little lower levels or, let's say, you throw in a little
22 DIC on top of what is going on so that you are not keeping
23 up your Factor VIII levels, then you need the ability to
24 give something besides DDAVP. It can be problematic

1 treating those patients. So, you know, when we use
2 concentrates we are not very comfortable stopping after one
3 or two days and then giving them a day off and seeing if
4 they don't bleed and then restarting concentrates. Yet,
5 that is what we do with DDAVP.
6 I am not against that in a way because it is
7 better than giving a blood product but it needs to be
8 considered in severe surgeries.
9 DR. FEDERICI: Just a brief reply. You are right.
10 The reason why I was presenting this data bout the registry
11 on vWD in Italy is the fact that we were a little amazed by
12 the fact that we found more type 1 treated with blood
13 concentrate. This comes up maybe with your observations
14 because, you know, if you have a very complicated situation
15 DDAVP can cover you 100%. Maybe you can have sort of mixed
16 treatment, DDAVP and Factor VIII concentrates.
17 DR. LUSHER: But I think what you are asking
18 though is, are such patients really good candidates for a
19 clinical trial with a new product? I mean, clearly, these
20 other groups are not muddied with other things going on.
21 DR. MACIK: I guess there is a subset of those
22 that are coming in for big surgery and you can pretty much
23 anticipate that DDAVP for two days -- let's say even a
24 bypass surgery for a moderately severe type 1 von Willebrand

1 is going to be a little tough to treat with DDAVP only, even
2 though it is a standard surgery, not complicated and is
3 anticipated to go well. That is a patient where you might
4 want a little bit more than just DDAVP coverage, depending
5 on how they go.
6 DR. MONTGOMERY: Certainly from a clinical
7 standpoint that is important. Those may not be the ones
8 though to include in a clinical trial. No one is trying to
9 withhold the treatment for those patients because they
10 surely need it, but they may not be the ones to best arrive
11 at what the dose is that we should be using.
12 DR. MACIK: I would concede that. However, these
13 are the patients, just as they found in Italy, that are most
14 often going to get concentrate when all is said and done
15 because the number of type 1 von Willebrand is so much
16 higher than all the 2's and 3's put together and, therefore,
17 have more surgeries, have more problems. So I would concede
18 that maybe for the first studies, although you might
19 consider, if you are going to treat major surgeries, that a
20 certain level of von Willebrand type 1's might still be
21 included.
22 DR. RICK: I think we have come to the third
23 question here and before Jeanne leaves, I would like to get
24 her feeling about what types of patients, indeed, would be

1 appropriate for a trial and perhaps the question of whether
2 they can have been pretreated with DDAVP, and then if you
3 have any feelings about the fourth question as well in terms
4 of dosing. As Dr. Pierce mentioned, we have had reports
5 today of very uniformly good responses and we don't really
6 know what the dose should be. Do you feel that we can treat
7 with those appropriate doses and half that appropriate dose
8 with, of course, an out to treat again with a higher dose
9 should bleeding occur?
10 DR. LUSHER: Well, in terms of dosage, you know,
11 one could argue in a number of ways. From what we have read
12 in the literature and what we have heard here today, it
13 seems like many patients are responding extremely well to a
14 dose of like 50 ristocetin cofactor units per kilo for
15 surgery. So, with these products that are in use and have a
16 track record now of being effective, but in order to do
17 studies to give them an indication for von Willebrand's
18 disease -- I guess it comes down to is it an ethical
19 question? We know that 50 U/kg works, say, with product X.
20 Is it really ethical to say, okay, we know that 50 works so
21 let's try 50 versus 10, to make a big difference? I think
22 Dr. Pierce said this morning if we are doing dosage studies
23 it should be a sufficient power, so in other words a
24 sufficient range, not like 50 versus 40 but something

1 substantially less. So one gets into the ethical dilemma
2 there, is that really something that we should be doing?
3 Or, when a product has been out there and used,
4 like some of the ones we have heard about today, and a
5 certain dosage, albeit empirically, has been used and seems
6 to be effective, should we not just evaluate that and make
7 sure that in a prospective clinical trial setting no one has
8 bleeding, or at least just an occasional person does?
9 DR. PIERCE: Earlier today there was a comment
10 about the correlation epidemiologically between Factor VIII
11 levels and coronary disease. It certainly seems that if
12 people are just treated sporadically and have sporadically
13 higher Factor VIII levels that is probably not a great
14 worry. But what about severe type 3 patients who really are
15 using the product on a prophylactic basis on a fairly
16 regular basis? There, there may well be an interest in
17 understanding what the minimum dosage interval should be or
18 the minimum dose, or to optimize the ratio of Factor VIII
19 activity to von Willebrand factor activity in order to
20 reduce the theoretical possibility of accelerated
21 atherosclerosis with these products.
22 DR. LUSHER: Right. There seems to be a variety
23 of types of products out there, as we have heard, in terms
24 of their Factor VIII content versus ristocetin cofactor,

1 probably all with different potential dosage regimes and
2 potential risks.
3 DR. PIERCE: The other point is that in
4 considering the ethical issue of looking at lower doses,
5 clearly, we need to do this relatively safely and not put
6 patients at undue risk. But are there certain clinical
7 circumstances, like dental extractions, that would be
8 relatively more safe to try a lower starting dose and then
9 give an increased follow-up dose if bleeding continued?
10 DR. LUSHER: The problem with dental extractions,
11 at least in my experience, is that local factors play such a
12 role: the operator, the person doing the extraction; the
13 local care of the wound site; whether or not one is allowed
14 to use anti-fibrinolytic agents in the clinical trial. So
15 that perhaps isn't the greatest one to be trying to find the
16 lowest dose, in my opinion.
17 DR. PIERCE: In cases where there are a lot of
18 confounding factors like that, do you think there would be a
19 role for, for example, randomizing patients to receive
20 fibrin sealants maybe in conjunction with anti-fibrinolytics
21 so that two dosage groups would both get that sort of
22 background standard of care, if you want to call it that,
23 although some of these products are not approved, and then
24 one treatment group would be randomized to additionally

1 being covered with product, and maybe at a couple of
2 different doses.
3 DR. MONTGOMERY: One comment I have about dental
4 surgery, and in my experience with hemophilia I think we
5 learned the lesson that to prevent a bleed from dental
6 surgery takes one treatment plus an anti-fibrinolytic. If
7 you have a breakthrough that occurs because you stopped
8 something too soon and it occurs at day four or five, try to
9 stop that one with one transfusion and anti-fibrinolytics
10 and it doesn't work. I think to subject patients to dental
11 surgery without coverage is not ethical if you know they are
12 a bleeder, just because you want to study it. To me, the
13 group that may have to get at what the minimal level is
14 would be to look at prophylaxis at two very dissimilar
15 levels to ask the question of when breakthrough bleeding is
16 going to occur. The problem is going to be -- if any of you
17 have ever prohylaxed against Factor VII deficiency with what
18 used to be prothrombin complexes, the effect far outlives
19 the plasma recovery, and that may well be a problem,
20 particularly since von Willebrand patients don't necessarily
21 bleed twice a week if untreated like, say, some hemophiliac
22 patients do.
23 DR. WHITE: I worry a little bit about the von
24 Willebrand factor link and thrombosis and atherosclerosis.

1 Just because a person with atherosclerosis or thrombosis has
2 a high von Willebrand factor doesn't mean that giving von
3 Willebrand factor will necessary cause thrombosis or
4 atherosclerosis. The high von Willebrand factor may simply
5 be an epiphenomenon from some other factor that is leading
6 to the thrombosis and atherosclerosis.
7 DR. KESSLER: As a physician treater, I would like
8 to make a plea that we not spend a lot of time discussing
9 minimal dosing but spend more time talking about optimal
10 dosing because if we have to wait to decide what minimal
11 dosing is, we are never going to be able to get this product
12 into the market. The comment was made that we don't know
13 what adequate minimal dosing is for hemophilia at this
14 point. So I think what we should really be looking at is
15 optimal dosing for the largest number of patients who have
16 von Willebrand's disease.
17 What I am most intrigued with is the issue of
18 thrombogenicity associated with this disease and the
19 treatment of this disease. Dr. Retzios reported one case of
20 thrombotic complications associated with a hemorrhoidectomy
21 that the investigator attributed to prolonged bed rest of
22 immobilization.
23 I think we have to remember several things. First
24 of all, whenever you design a clinical trial and you

1 normalize these individuals with their von Willebrand and
2 their Factor VIII level, I think you are going to have to
3 design the trial in such a way that you actually prophylax
4 these individuals the same way you might prophylax a normal
5 individual who is undergoing hip, knee, or any other pro-
6 coagulant related surgery.
7 From what Dr. Retzios presented, it almost makes
8 me wonder whether we should be looking at von Willebrand
9 patients for other inherited defects, such as Factor V
10 leiden, in view of the recent reports that have indicated
11 that individuals with hemophilia A who also have co-
12 inheritance of Factor V leiden may actually have decreased
13 bleeding as part of the course of their disease. I am
14 wondering whether if we looked for Factor V leiden defects
15 in von Willebrand patients we would see a similar type of
16 decreased risk for bleeding and an increased risk for
17 thrombotic complications that we have to be careful about
18 prophylaxing for.
19 DR. JOIST: I was going to say exactly what Craig
20 just pointed out. We don't know what the minimal dose in
21 hemophiliacs in various indications is. I think at this
22 point we are continuing to use an off-label drug, and we are
23 expecting other physicians to use an off-label drug in von
24 Willebrand disease that we know is effective in control of

1 bleeding in certain patients with von Willebrand's disease.
2 I think there is certainly more research that is needed to
3 look more carefully at what the minimal effective doses are,
4 and that is going to be very difficult given the number of
5 patients available, the different types of bleeding
6 situations that we can encounter, and so forth. But I think
7 it is time that at least one or two of these preparations
8 are approved for clinical used so that we do not any more
9 use them off-label, and that we then go on and do additional
10 studies to define our treatment modalities.
11 DR. PIERCE: Can we design a study in such a way
12 as to tell the difference between a good dose and a better
13 dose? I agree with you, we don't necessarily have to set up
14 the hurdle of determining the minimum effective dose pre-
15 approval of any product, but the idea is, you know, what are
16 our options for trial design? What kind of control groups
17 are feasible and appropriate to use? How do we judge one
18 product against another?
19 DR. JOIST: Well, I think these are very difficult
20 questions and they can't be answered here in about two or
21 three minutes. You have to look at different surgical
22 procedures. You have to look at different injury
23 situations; dental surgeries. These are all different. I
24 think we are aided by experience in hemophilia, knowing

1 pretty well what doses we can use that are reasonably
2 effective, and I don't think you can come up with an overall
3 cutoff and say, well, 50 units is good for everybody. But I
4 think a group of experienced treaters could come up and help
5 the FDA to establish, in a preliminary sense, an adequately
6 preliminary sense, what the recommended doses should be at
7 this sort of stage of experience for various indications.
8 It doesn't have to be too specific. I think that could be
9 done based on the knowledge that we have and the experience
10 that we have.
11 DR. MONTGOMERY: If I could make one comment, we
12 shouldn't lose sight of the fact that normal patients with
13 surgery have elevated von Willebrand factor and Factor VIII.
14 So that necessarily patients that are 200% Factor VIII or
15 300% that may, in fact, be physiologic.
16 DR. RETZIOS: Well, I would like to add also that
17 the issue of number of doses is that ATC93-01, the trial
18 that we designed at Alpha, did attempt to answer that
19 question within safety parameters that were acceptable.
20 Actually, when we designed the study in the beginning of
21 1993, all we had to go by were the studies by Dr. Manucci,
22 and Dr. Manucci had, indeed, dosed the patients at about 60-
23 70 units of ristocetin cofactor per kilogram. We then built
24 two dosing groups, 40 and 60, and we would have infused more

1 patients at 60 had the 40 ristocetin cofactor not reacted as
2 well as it did. So, the point is that it is very difficult
3 sometimes to obtain IRB approval and to go back and say,
4 well, now we would like to look at 20 but it is likely, from
5 our results so far and possibly the dosing relationship that
6 we have, that some of the patients are really not going to
7 respond very well. I don't think that the IRB would
8 probably allow us to perform such a study.
9 DR. FEDERICI: I have some comments. I want to
10 comment about prophylactic dose for dental extractions and
11 side effects, namely thrombotic. We have a lot of
12 experience with prophylactic dosage. In the last twenty
13 years I think that I have seen many times type 3 vWD with
14 hemarthrosis. So we usually give them concentrate. In the
15 old days we gave, unfortunately, cryo. After the
16 concentrates were available -- actually, the patients are
17 now able to treat themselves at home. They call the center
18 and they say we have hemarthrosis. What do we have to do?
19 So they are used to treating themselves every other day with
20 a dosage of about 30, 50 units every other day for at least
21 one or two weeks until the problem of hemarthrosis is
22 solved.
23 The problem with dental extraction, I agree with
24 Bob -- Bob knows how these kind of things work. So, if you

1 are relying on a good dental surgeon -- we have a sort of
2 good retrospective analysis. We wanted to mimic what some
3 people were proposing in a meeting last year, sort of
4 treating on demand. We went to some of these dental
5 extraction patients without concentrate, being very careful,
6 of course. We have been following some of the type 3 for
7 just one dental extraction, and it was enough to have a good
8 surgeon and washing with transanamic acid in order not to
9 have problems. Maybe we don't have enough data, but when we
10 have more data we will publish this data. So we have to
11 know that.
12 Then the side effects, thrombotic, in my twenty
13 years experience I have never seen any thrombotic events in
14 vWD. The reports by Retzios with Alphanate is a unique
15 experience. I have a patient in Milano. That patient was
16 very ill. He was not diagnosed in our center. He was
17 followed by our center because he moved to Milano. He had
18 HIV infections and he had the most important thing, chronic
19 hemarthrosis, and he got DVP, DVP in that leg. I am
20 concerned about not having too much Factor VIII C around,
21 but I am not that concerned because these were local
22 situations, local problems of that patient. You know that
23 HIV can have sort of activation of endothelial cells. We
24 don't know why. But the most important thing he got was

1 hemarthrosis, chronic hemarthrosis in that leg and in his
2 knees. So the venous circulation could be influenced by the
3 fact that he was operated on. Post-operation he could not
4 move his leg, as other type 2 vWD. So we have to realize
5 that. By chance, we also checked in that patient Factor V
6 leiden and it was negative.
7 DR. SCHWARZ: Guidelines for the diagnosis and
8 management of von Willebrand's disease -- when was that
9 published? A few weeks ago, in the supplement of
10 Hemophilia, prepared by the Hemophilia Center in the United
11 Kingdom. Everything is written here. The question is
12 should the community stick to these published guidelines or
13 are we discussing new issues? I mean, it is really very
14 recent. So my question would be for surgery for patients
15 with von Willebrand's disease, von Willebrand factor
16 concentrates, only concentrates containing von Willebrand
17 factor should be used. Preoperatively Factor VIII C levels
18 should be raised to 100%. This would also raise von
19 Willebrand factor level to above 100%. Treatment may be
20 required 12 hours later, etc., etc. Factor VIII activity
21 levels pre- and post-treatment should be assayed. Von
22 Willebrand factor activity and antigen levels -- activity
23 and antigen should be assayed pre- and post-treatment for
24 the first three treatments so as to follow a more informed

1 plan of therapy.

2 I mean, this is an important document also on the
3 liability issue. Do we have to stick to this, or what is
4 your feeling? We have one of the authors here.

5 DR. PASI: Yes, I was one of the people that was
6 involved in writing this document. I am John Pasi, from
7 Royal Free, London.

8 I would like to say that many issues that have
9 been mentioned today at this Workshop were actually thought
10 about at the time we were writing that document, and we
11 wrote that document to be the broadest type of document that
12 would cover practical procedures and looking after patients
13 at the time. I don't think it is cast in stone by any
14 means, and I think it just provided a practical solution to
15 the treatment of patients with von Willebrand's disease.

16 DR. WHITE: That would have been my comment too.
17 I think that document was conceived as a guideline for
18 treatment document. The questions that are being discussed
19 here are slightly different. It is how do you determine
20 whether a product is working or not? They are related
21 questions but they are not necessarily identical questions.
22 So it is not unrealistic to re-raise some of those issues
23 and say is this the best way to do it? I think those are
24 fair questions.

1 DR. RICK: I think the hour is late. We haven't
2 dealt with the last question. Mark, I need to have some
3 guidance from you whether we should thank all the
4 participants in the audience or whether we should try for
5 one more question.
6 DR. WEINSTEIN: (Not at microphone; inaudible)
7 DR. RICK: The answer was let us try to go on to
8 talk a little bit about data should be collected in whatever
9 trial is designed here. Let me ask Ross to elaborate on
10 that just a bit.
11 DR. PIERCE: Sure. So, we are really talking
12 about some of the nitty-gritty of the pros and cons of
13 different clinical trial design options. Thinking in terms
14 of how each product that is presented to the FDA should
15 ideally in the future be evaluated, what choice of control
16 group is most appropriate? Patients as their own controls?
17 What kind of documentation of previous episodes prior to any
18 therapy should be available? In my presentation I mentioned
19 historical controls to normal individuals. We heard about a
20 trial today where clinical investigators were asked to fax,
21 24 hours before the patient went to surgery, what they would
22 guess a normal non-bleeding disorder patient would lose in
23 the way of blood for that particular surgical procedure.
24 Crossover trials comparing, for example, products that are

1 devoid of Factor VIII activity versus those that have the
2 combination of the two clotting factors, as is being planned
3 in Europe. What clinical endpoints are considered most
4 informative as a primary efficacy endpoint and as additional
5 endpoints that can buttress our confidence in the outcome of
6 a trial, and how can we fold together the design of trials
7 to have us understand the relationship between
8 pharmacokinetics and clinical activity to really get at what
9 was mentioned before, how do we optimize therapy for the
10 benefit of patients and to aid the treating physician.
11 DR. MENACHE: I would like to ask a question
12 because I am a little bit confused. I heard a control
13 population. My understanding is that for a control I should
14 have a group which is treated and a group which is not
15 treated. Now, I have heard about crossover studies with
16 different products. We don't have a licensed product. What
17 are we going to cross it with? Cryoprecipitate? In Europe
18 it is licensed. What choice do we have here? Crossover
19 studies with what?
20 DR. WHITE: Well, I think that is where I was
21 getting stuck too, on the control. I don't think it is
22 ethical to do a cryoprecipitate control.
23 DR. MENACHE: No.
24 DR. WHITE: I don't think anybody would feel that.

1 I think the control that Ross is talking about is more a
2 control in which where an individual undergoing a procedure
3 there is an attempt to try and compare that individual with
4 some theoretical control of a patient who is undergoing the
5 same procedure who doesn't have a bleeding disorder.
6 I think any time you ask a surgeon how hemostasis
7 is and you give him four or five choices, excellent, good,
8 fair, poor and none, what he is basically doing is, in his
9 mind, comparing that with historical controls.
10 DR. MENACHE: Right.
11 DR. WHITE: I don't really see a way to do a
12 control here. I think it would be nice to do controls
13 because, unlike hemophilia where if you take a severe
14 hemophiliac through a procedure you are pretty sure you are
15 going to take bleeding if you don't treat him, here
16 sometimes you are not quite as sure.
17 DR. MENACHE: Have you tried? A severe von
18 Willebrand surgery with no replacement therapy? It works?
19 I mean, who would try? You wouldn't.
20 DR. WHITE: Well, I am thinking because I think
21 that is a good question and that is what I am implying, but
22 I am sure severe von Willebrand patients have had surgery
23 without coverage. I am sure Glanzmann's patients have had
24 surgery without coverage, and I am sure hemophiliacs have

1 had surgery without coverage.
2 DR. MONTGOMERY: But not with informed consent.
3 DR. WHITE: Not with informed consent.
4 (Laughter)
5 DR. WHITE: I am not advocating that. Don't
6 misunderstand me, I am not advocating that, I am just saying
7 that the thought does go through your mind how do you
8 compare this with something? I think that is what Ross is
9 asking. But my bottom line -- I was looking over those
10 questions, and the one I was really stuck on was the first
11 one and I don't see a way to do a control. I think the FDA
12 has to assume that although there is no licensed product
13 here, there is a standard of care which is being performed,
14 which provides patients with von Willebrand's disease with a
15 treatment. Treatments have to be compared against that
16 standard of care, in my opinion. You can't go back and say,
17 well, what we have done for the last twenty years doesn't
18 count; we have to do controlled studies; you have to simply
19 compare it with a control. I am not saying the FDA is
20 advocating a no treatment or some other type of control. I
21 think they probably agree with what I just said, but to just
22 put that out there, I think that is what the control has to
23 be. It has to be what our standard of care has been up to
24 now whether it is approved for that use or not.

1 DR. PIERCE: One very specific thing that I would
2 like to throw out though is actually question 4B, and that
3 is treatment duration. As we heard the results of the
4 American survey, I compared what I heard about the number of
5 treatments in surgeries where it was uncommon for patients
6 to get more than five infusions, if I understood correctly,
7 to the average number of infusions used with the French LFB
8 product in their patient series, which was around 17
9 infusions for major surgery, and I wonder even with a
10 standard of care product if, at some point, there isn't room
11 to do a trial that looks at whether an outcome can be good
12 with randomizing patients to one minimum number of infusions
13 and better, lower incidence of rebleeding, if they receive a
14 larger number of infusions.
15 DR. MENACHE: I would like to make one comment.
16 When we first developed the protocol to evaluate the LFB-
17 manufactured product, I had a meeting with the clinical
18 investigators to see at what level they wanted the Factor
19 VIII prior to surgery, and to calculate what amount of
20 product we should do. Bob was attending that meeting and
21 there were several investigators, and they would not budge
22 below 100%. Finally, with a lot of difficulty and
23 discussion we agreed on 80%. In France, with this same
24 discussion, 60% was enough.

1 So, what I am trying to say is that, number one,
2 no one knows what is the minimum level required for
3 hemostasis. No one is going to take the risk of knowing,
4 neither the physician nor the patient. In the United States
5 we treat patients with much higher dosage than in Europe.
6 It has always been the case. We were richer. We had more
7 products. We used more. Now it is a little bit different.
8 But it is extremely difficult to decide ahead of time. If
9 you discuss it with physicians, they don't want a level of
10 less than 80%. On what basis? I don't know. But they
11 don't want to take the risk of having a problem with a
12 patient. I just wanted to make this comment.
13 DR. RETZIOS: I would like to add to this. It is
14 the same with the Alphanate study, 100% of Factor VIII was
15 deemed the minimum limit before proceeding with surgery. If
16 the patient actually did not achieve 100% Factor VIII prior
17 to surgery, the surgery was aborted. It didn't happen in
18 any of our cases but that is the provision of the protocol,
19 and it came through the meetings with the investigators.
20 Regarding a possible control study, I would like
21 to offer a historical note. About the beginning of 1994 we
22 were approached by CBER to run a control study against
23 cryoprecipitate. We did try, actually, to even get the
24 safest cryoprecipitate we could possibly get our hands on.

1 We aged the cryoprecipitate; we did all kinds of PCR work.
2 However, we couldn't find a single investigator who was
3 willing to enter in a study like that. Plus, the study had
4 other complications -- how could you match the dose of the
5 cryoprecipitate to the dose of Alphanate? You probably had
6 to dose almost 1.5 liters of cryoprecipitate in order to
7 achieve the same dose of Alphanate. So, nobody was willing
8 to proceed with this control study.
9 DR. MONTGOMERY: I think somewhere in the course
10 of doing these studies to try to get at the question of
11 minimal effective dose to prevent bleeding it may require
12 prophylaxis in patients that bleed frequently, and it is a
13 totally different study. Given that the standard of care,
14 at least in most places, is not yet prophylaxis, then being
15 able to do something on different levels of prophylaxis
16 could be useful. If you actually could determine that
17 prophylaxis with 10 units -- let's just be extreme -- 10
18 U/kg/week as opposed to 20 U/kg every other day, or
19 something, in someone that maybe bleeds several times and is
20 not on prophylaxis might be able to get at the question of
21 are there really levels, other than zero, at which bleed in
22 such patients.
23 DR. RICK: Are there other comments? If not, we
24 certainly thank all of the participants, and I would like to

1 thank the panel particularly, and we stand adjourned.

2 (Whereupon, at 5:25 p.m., the Workshop adjourned.)