

1       STATE OF INDIANA       )       IN THE MARION SUPERIOR COURT  
2       COUNTY OF MARION     )       SS: CIVIL DIVISION       ROOM ONE  
                                  )       CAUSE NUMBER 49D01 9312 CT1236

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4       [GRO-A], and [GRO-A] personally       )  
5       and as surviving guardians of       )  
6       [GRO-A], Deceased,       )  
  )  
7       Plaintiffs,       )  
  )

8       vs.       )

9       ARMOUR PHARMACEUTICAL CORP.,       )  
10      CUTTER LABORATORIES,       )  
11      DIVISION OF MILES, INC.,       )  
12      BAXTER HEALTHCARE CORPORATION,       )  
13      f/k/a Hyland Therapeutics,       )  
14      Division of Travenol       )  
15      Laboratories, Inc., and       )  
16      ALPHA THERAPEUTICS CORPORATION,       )  
17                                       )  
18      Defendants.       )

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22                                       TRANSCRIPT OF TESTIMONY OF  
23                                       PETER FERNANDES

24

25

26       Before the Honorable

      Anthony J. Metz, III, Judge

      February 12 and February 13, 1997

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A P P E A R A N C E S

Questions read by: Larry Jackson, Esq.  
301 Massachusetts Avenue  
Indianapolis, Indiana, 46204

Answers read by: Henry Price, Esq.  
301 Massachusetts Avenue  
Indianapolis, Indiana, 46204

For Defendant Charles Goodell, Esq.  
Commerce Place  
One South Street, 20th Floor  
Baltimore, Maryland, 21202

Mary Beth Hall, Court Reporter

1       **DEPOSITION OF PETER M. FERNANDES**

2       Q.   All right.  And then your deposition was scheduled  
3           for today.  is that correct?

4       A.   Right.

5       Q.   All right.  Tell me the contact you've had with  
6           lawyers for Bayer, including Mr. Dever, between  
7           the time you received the subpoena and today.

8       A.   I met with Mr. Dever on Wednesday -- no, I think  
9           Thursday afternoon or Thursday evening really for  
10          a few hours, and we actually went over my lab  
11          notebooks, and which I actually shared with him an  
12          explanation of actually some of the experiments I  
13          had done.

14                We also met -- this probably was a couple of  
15          hours, from 4:30 to around 6:30 in the evening.  
16          We met the next day, which was yesterday, from  
17          about 9:30, maybe about an hour and a half, 10:30,  
18          11:00, just completing a review of my notebooks.  
19          And at that point I left, until this morning.

20      Q.   Was anyone, besides Mr. Dever, present with you  
21          during those meetings?

22      A.   No.

23      Q.   Were any documents reviewed, other than your  
24          laboratory notebooks?

25      A.   No, it was primarily and only my lab notebooks.

- 1 Q. Had you read other depositions besides the ones  
2 you identified earlier?
- 3 A. No.
- 4 Q. Had Mr. Dever told you what any of the other  
5 witnesses in the litigation have testified to up  
6 to this point?
- 7 A. I can't recall any kind of that information.
- 8 Q. Well, for example, did he relate any of the  
9 testimony of Dr. Mann, one of the experts for  
10 Bayer?
- 11 A. No.
- 12 Q. Did you do any medical or scientific research or  
13 review any articles in preparation for coming here  
14 and giving testimony?
- 15 A. No.
- 16 Q. Nothing? You didn't read anything?
- 17 A. Medical research? No.
- 18 Q. Scientific research?
- 19 A. I haven't read any scientific research  
20 specifically addressing this particular work.
- 21 Q. Have you gone back and reviewed the patent that  
22 you hold concerning the pasteurization of plasma  
23 proteins?
- 24 A. I went back, yes, and I had looked at a patent,  
25 but I didn't go through it line by line, I think.

1 I just went to make sure that I recollect it in  
2 general.

3 Q. Right. When did you do that?

4 A. I think last night. Yesterday morning. I'm  
5 sorry.

6 Q. Is the patent here?

7 A. I don't know. It's not with me.

8 Q. Are we talking about the patent that was granted  
9 in the year 1984?

10 A. Yes, I think it's the patent in which I played the  
11 primary role.

12 Q. Were there any other patents that you reviewed?

13 A. No.

14 Q. You have been kind enough to supply us with a copy  
15 of your CV, which I have marked as Exhibit 1. I  
16 understand that you have another copy of it in  
17 front of you. Would you just confirm that the  
18 copy that I have marked is the same as the copy  
19 that you have in front of you there?

20 A. Yes, it is.

21

22 **MR. JACKSON:** Plaintiffs would offer the  
23 curriculum vitae of Dr. Fernandes into  
24 evidence.

25 **THE COURT:** What's the number?

1                   MR. JACKSON: It's Plaintiffs' 970.

2                   MR. GOODELL: No objection.

3                   THE COURT: 970 is admitted.

4

5           DEPOSITION OF DR. FERNANDES RESUMED

6           Q.    Okay. As I understand it, you graduated in 1970  
7                   from the Indian Institute of Technology in India.  
8                   Is that correct?

9           A.    That's correct.

10          Q.    And when did you come to the United States?

11          A.    Very close after that. I came to the U.S. in  
12                  August of '79. Seventy. I'm sorry.

13          Q.    Of 1970?

14          A.    Yes.

15          Q.    And did you immediately begin studies at Rutgers  
16                  University in furtherance of obtaining a Ph.D?

17          A.    Yes, I did.

18          Q.    And it my understanding here that you obtained  
19                  your Ph.D in 1975 in Chemical and Biochemical  
20                  Engineering. Is that correct?

21          A.    That's correct. Yes, it is.

22          Q.    Can you give us, if you don't mind, a general  
23                  statement as to what chemical and biochemical  
24                  engineering are, please? By the way, are they  
25                  separate fields or are they a combined field?

1       A.   Well, they are actually becoming more and more  
2           integrated into chemical and biochemical  
3           engineering, but in the early seventies the option  
4           of basically biochemical engineering was a new  
5           field, and there were very few universities  
6           offering this particular option, and there were  
7           even fewer of them offering it as a combination.  
8           So that was the reason why I picked Rutgers.

9           Basically, biochemical engineering is an  
10          application of engineering principals to the  
11          biological processes. So if one was interested in  
12          fermentation, trying to grow bacteria or mammalian  
13          cells on a large scale, there are issues of mass  
14          transfer and heat transfer and rheology and a lot  
15          of engineering functions, and those were programs  
16          that Rutgers tried to combine with microbiology  
17          and biochemistry to try and get a hybrid  
18          understanding of the field.

19       Q.   Is biochemical engineering different than protein  
20           biochemistry?

21       A.   Yes, it is very different because, in a sense,  
22           there is an overlap, but basically protein  
23           biochemistry spends a lot of effort looking at  
24           mechanisms. What we were looking at primarily is  
25           the application of those on a large scale.

- 1 Q. Right. In other words, you don't, I'm sure,  
2 consider yourself a protein biochemist. Is that  
3 correct?
- 4 A. No, I don't consider myself a protein biochemist,  
5 though as part of the biochemical engineering  
6 program, we had to take a few courses in the life  
7 sciences, which included biochemistry and  
8 microbiology.
- 9 Q. Right. But I take it significantly less than you  
10 would have been required to take if you were  
11 trying to get your Ph.D in protein biochemistry?
- 12 A. Yes, significantly less.
- 13 Q. Now, did you have to do a thesis as part of your  
14 Ph.D program?
- 15 A. Yes, I did.
- 16 Q. Is the topic of your thesis listed on your CV?
- 17 A. No, it's not.
- 18 Q. Do you have access to it?
- 19 A. I don't have access to the actual document, but I  
20 will let you know what it's all about if you are  
21 interested.
- 22 Q. Well, I'm interested, but maybe only curiously  
23 interested. Why don't you tell me what the topic  
24 of it was.
- 25 A. Well, the actual topic was the mathematical

1 modeling of an immobilized multi-enzyme reactor  
2 system, and in very, very simple terms it meant  
3 trying to use enzymes in a large scale industrial  
4 reactor, and we were very concerned with  
5 instability of enzymes in an environment in which  
6 we were pumping gases and liquids.

7 So the essential work was trying to write a  
8 mathematical model on how the system would work,  
9 go to the labs and be able to show that I could  
10 experimentally validate that model. That  
11 essentially was my thesis.

12 Q. And were you able to do that?

13 A. I was able to do it with varying degrees of  
14 success.

15 Q. What enzymes were you working with?

16 A. The enzymes I was working with, one was glucose  
17 oxidase and the second was catalase, and we tried  
18 to couple the two enzymes together.

19 Q. Were you concerned about the stability of the  
20 enzymes during the reactor process?

21 A. Yes.

22 Q. And how were you able to ensure the stability of  
23 the enzymes?

24 A. One of the things that we had been doing at  
25 Rutgers University for many years prior to my

1           working on the program was to look at several ways  
2           to immobilize enzymes, and we felt that was an  
3           efficient way to keep these enzymes in active  
4           form.

5                   Immobilization really means fixing these  
6           enzymes into an insoluble bead or a matrix, and so  
7           much of my work went into trying to find the best  
8           way to get the glucose oxidase to be immobilized  
9           on this matrix and still exhibit active property,  
10          and did the same thing with catalase, and then  
11          tried mixing the two in a single reactor and  
12          looking at yield.

13       Q.    Okay.   Current, that is up to the time you  
14              completed your Ph.D program in 1975, had you ever  
15              worked with plasma proteins?

16       A.    No.

17       Q.    I mean, were you familiar, do you believe -- or  
18              did you at least have knowledge that there were  
19              two plasma proteins called Factor VIII and Factor  
20              IX?

21       A.    No.   I had no idea about the plasma fractionation  
22              industry period.

23       Q.    Had you ever done any work, current again up  
24              through the year 1975, with attempting to  
25              stabilize any proteins?

- 1 A. Not --
- 2 Q. You talked about the immobilization?
- 3 A. Not any more than the concept of immobilization.
- 4 But no more than that, no.
- 5 Q. Had you done any research up to 1975 in terms of
- 6 attempting to identify in your own mind general
- 7 stabilizers that were available and had been
- 8 discussed in the literature for purposes of
- 9 stabilizing proteins?
- 10 A. No.
- 11 Q. Had you ever done any work attempting to purify
- 12 proteins?
- 13 A. I had done some limited work in terms of isolating
- 14 enzymes from yeast as part of my program in trying
- 15 to get enzymes to run these reactors.
- 16 Q. Did you have any knowledge whatsoever of
- 17 purification technology that could be used with
- 18 plasma proteins, current to the year 1975?
- 19 A. None of the work I had done at Rutgers was really
- 20 geared towards separation of plasma proteins, but
- 21 I did use the methods of ion exchange and gel
- 22 filtration as methods to purify the enzyme I was
- 23 interested in for my thesis.
- 24 Q. Right. How about working with polyethyleneglycol
- 25 or glycine or lysine or any of those chemicals?

- 1 Had you ever done any work with those?
- 2 A. Not with those specifically.
- 3 Q. And I take it specifically you never did any work
- 4 at all trying to purify plasma proteins. Is that
- 5 right?
- 6 A. That's right.
- 7 Q. Do you know up to the year 1975 whether you had
- 8 read any literature of any kind having to do with
- 9 the purification of plasma proteins?
- 10 A. No.
- 11 Q. Were you current in the field up to the year 1975,
- 12 and I'm talking about the field of protein
- 13 stabilization, had you reviewed the literature?
- 14 Were you aware of the body of scientific knowledge
- 15 having to do with the topic of protein
- 16 stabilization?
- 17 A. No.
- 18 Q. Had you ever worked in any context, again up to
- 19 the year 1975, with model viruses or viruses of
- 20 any kind?
- 21 A. No.
- 22 Q. Had you ever done any viral inactivation work of
- 23 any kind up to the year 1975?
- 24 A. No.
- 25 Q. Had you worked in any context with hepatitis?

1 A. No.

2 Q. Is it fair to say then that up to the year 1975  
3 you essentially had no experience whatsoever in  
4 the field of viral inactivation?

5

6 MR. PRICE: I think you may have misread  
7 a word, Mr. Jackson. Expertise.

8 MR. JACKSON: Okay. "You essentially  
9 had no expertise..." I said "experience".

10

11 **DEPOSITION OF DR. FERNANDES RESUMED**

12 Q. Essentially no expertise whatsoever in the field  
13 of viral inactivation?

14 A. That is right.

15 Q. Or any expertise, for that matter, in the field of  
16 plasma proteins?

17 A. That is right.

18 Q. Your CV indicates that in the year 1975, I guess,  
19 you had the pleasure of joining Armour  
20 Pharmaceutical in Kankakee. Is that right?

21 A. Yes, I did.

22 Q. And you worked there from January of 1975 to May  
23 of 1976. Is that correct?

24 A. That's correct.

25 Q. That's what your CV says. I'm just reading the

1           dates. That was at their manufacturing facility.

2           Correct?

3       A.    Yes. That was in Illinois in Kankakee.

4       Q.    All right. We are certainly going to have an  
5           opportunity to review your work at Armour by  
6           looking at documents similar to what we are doing  
7           today. But give me a general idea, if you can,  
8           what your duties and responsibilities were at  
9           Armour Pharmaceutical.

10      A.    Well, when I actually joined Armour, I was in the  
11           manufacturing support organization, and one of the  
12           areas that I was involved with was looking at the  
13           complete plasma fractionation process. Clearly at  
14           that time we were dealing with plasma being  
15           obviously a very rare resource, trying to find  
16           ways to improve it, trying to see where we can  
17           minimize wastage, if there were any, or try to  
18           improve it.

19                So the methods that I was looking at was  
20           looking at how they manufacture albumin and gamma  
21           globulins, specifically looking at how they  
22           schedule operations, is there a way through  
23           optimal running of the process one could improve  
24           the general quality of the protein. There was a  
25           concern, again dealing with the fact that we got

1           limited resources in the sense that the plasma was  
2           not widely available and it's a very important  
3           resource, we were trying to look at changing the  
4           manufacturing process from a batch process to a  
5           once through continuous process, and that was  
6           utilizing my background in engineering. Rather  
7           than going from the first step to the second to  
8           the third, we were trying to have a continuous  
9           process. You feed in plasma in one end and pick  
10          out albumin from the opposite.

11        Q.    So just to kind of put it in layman's terms so  
12              that the Jury would understand, you were tinkering  
13              with aspects of the manufacturing process to  
14              improve the quality of the ultimate products that  
15              were being made from the plasma. Is that a fair  
16              statement?

17        A.    We were trying to improve the manufacturing  
18              process, yes. One of the aspects was quality,  
19              clearly.

20        Q.    Right. Now, I'm curious, Dr. Fernandes. Who told  
21              you that the plasma that was being used in the  
22              manufacture of these products was in short supply?

23        A.    Well, this is from my personal observations as I  
24              joined the company. I was aware of the fact that  
25              plasma is not readily available, human plasma is

- 1 not readily available. It was a personal  
2 assumption on my side.
- 3 Q. Well, did somebody at Armour tell you that they  
4 were having difficulty collecting plasma to meet  
5 the manufacturing needs of the company?
- 6 A. (no audible response)
- 7 Q. Would you like the question read back or do you  
8 remember it?
- 9 A. I remember the question, and the answer is just  
10 from my own personal observation, it wasn't -- I  
11 was not informed by anybody specifically. I was  
12 told when I went to Armour and on my first couple  
13 of days looking at new areas to work on, this is  
14 an opportunity for you to look at improvements in  
15 terms of the process, because we are dealing with  
16 a very precious resource, and it was from that I  
17 assumed that this is something that you should  
18 take care of.
- 19 Q. Did you know that Armour was collecting the plasma  
20 from paid commercial donors?
- 21 A. I didn't know at that time.
- 22 Q. How did you protect yourself against infection  
23 with hepatitis during the time period that you  
24 were working at Armour?
- 25 A. I actually was very careful. I was aware of the

- 1 risk, and I practiced good laboratory practices.
- 2 Q. When you came to Armour, did someone there explain
- 3 to you that this plasma that you were working with
- 4 can cause you to get hepatitis if you are not
- 5 careful?
- 6 A. Yes, I think as part of my orientation program for
- 7 the first couple of days that I was -- yes, the
- 8 risk associated with plasma processing was
- 9 mentioned to me.
- 10 Q. Did people at Armour explain to you that there had
- 11 been manufacturing personnel who had actually
- 12 gotten hepatitis from working with these plasma
- 13 products?
- 14 A. I can't recollect whether they had said anything
- 15 to that effect or not.
- 16 Q. Did you take any prophylactic medication yourself
- 17 to guard against being infected with hepatitis?
- 18 A. Not when I was at Armour.
- 19 Q. You did later when you were at Cutter?
- 20 A. When I joined Cutter I began taking an IM shot of
- 21 gamma globulin intramuscular.
- 22 Q. And correct me if I'm wrong, but the purpose of
- 23 taking that medication, I presume, was to attempt
- 24 to ward off any hepatitis infection if you were
- 25 inadvertently exposed. Is that correct?

- 1       A.    It was actually part of the orientation program  
2            when I joined Cutter.  That was one of the  
3            policies there was that I was given an option of  
4            saying whether I'd like to have IM gamma  
5            globulins, and I chose to take it.
- 6       Q.    Well, why did you understand you were being  
7            offered the IV gamma globulin medication?  Was it  
8            IV or IM?
- 9       A.    It was IM at that time.
- 10      Q.    All right.  Why did you understand that you were  
11            being offered that medication?
- 12      A.    I think there was an awareness in the company that  
13            there was a real risk that was available, even  
14            though there were measures taken by the company to  
15            control the level of hepatitis either through the  
16            system in which they were receiving the plasma.  
17            But I saw it in terms of good common sense on my  
18            part to take it.
- 19      Q.    Did most of your colleagues at Cutter also take IM  
20            gamma globulin shots?  By "colleagues," I'm  
21            talking about persons in the laboratory.
- 22      A.    I can't recollect whether everybody did or not.  
23            There are probably people that did.  I'm not aware  
24            specifically.
- 25      Q.    Well, getting back to your work at Armour, is it

- 1 fair to say that most of the work at Armour during  
2 the period January of '75 to 1976 was working with  
3 the gamma globulin products and albumin?
- 4 A. There was one excursion in that effort, and that  
5 was toward the end of my stay there was some  
6 problems dealing with the freeze-drying of Koate.  
7 It wasn't called Koate at Armour.
- 8 Q. It was Factor VIII at Armour.
- 9 A. Factor VIII. So I actually played a little role  
10 in terms of helping, from a manufacturing  
11 standpoint again, looking at ways of optimizing  
12 the freeze-drying process. But that was my only  
13 exposure to anything other than albumin and gamma  
14 globulin.
- 15 Q. All right. So all the questions I am getting  
16 ready to ask you have to do with a period that you  
17 were working at Armour up to May of 1976. Is it  
18 fair to say that up to that time you had not  
19 directly worked in the laboratory with Factor VIII  
20 or Factor IX?
- 21 A. That's true.
- 22 Q. Had you ever performed a Factor VIII assay?
- 23 A. No.
- 24 Q. Had you ever performed a Factor IX assay?
- 25 A. No.

- 1 Q. Had you ever tried to work up purification schemes  
2 for Factor VIII or Factor IX?
- 3 A. Not during my stay at Armour.
- 4 Q. And had you done any work with protein  
5 stabilization of any kind, current to May of 1976?
- 6 A. No.
- 7 Q. Were you asked by Armour to help solve protein  
8 stabilization of Factor VIII or Factor IX while  
9 you were at Armour, current again to May of 1976?
- 10 A. No.
- 11 Q. Had you worked with model viruses or hepatitis or  
12 any other viruses as part of your duties at  
13 Armour?
- 14 A. No.
- 15 Q. Who were you reporting to at Armour?
- 16 A. I was reporting to Dr. Don Meter, M-E-T-E-R.
- 17 Q. Was Dr. Feldman at the company at that time?
- 18 A. Yes, I think he had joined at that time.
- 19 Q. But he was not in the part of the company that you  
20 were in. Is that correct?
- 21 A. No.
- 22 Q. He was in the research end and you were in the  
23 manufacturing end. Is that correct?
- 24 A. Yes, that is right.
- 25 Q. Who was the head of manufacturing at Armour during

1 the period January of 1975 to May of 1976?

2 A. I don't recollect.

3 Q. When you left Armour in May of 1976, did you  
4 consider yourself an expert on Factor VIII?

5 A. No.

6 Q. Did you consider yourself an expert on Factor IX?

7 A. No.

8 Q. Current to the year May of 1976, what professional  
9 organizations, if any, did you belong to?

10 A. I was primarily involved in the American Institute  
11 of Chemical Engineering, which was my primary  
12 affiliate body.

13 Q. Did you belong to any other professional societies  
14 that had as a component of their work a study of  
15 the coagulation sciences?

16 A. No.

17 Q. Have you ever belonged to any of those  
18 organizations?

19 A. No.

20

21 MR. JACKSON: Starting on line 13 of  
22 page 33.

23

24 DEPOSITION OF DR. FERNANDES RESUMED

25 Q. How is it that you decided to leave Armour and

- 1 join Cutter?
- 2 A. It was very interesting because, as I mentioned, I  
3 had been doing a lot of work in terms of gamma  
4 globulins and albumin, and for other reasons, we  
5 felt this was an ideal time to make a move, and I  
6 happened to meet an individual at Armour whose  
7 name was Al Pappenhagen who said, "Look, you have  
8 been thinking about moving, why don't you try  
9 Cutter," which I did and joined subsequently.
- 10 Q. Did Mr. Pappenhagen, Al, come with you at that  
11 time to Cutter or did he come later?
- 12 A. I think he came later.
- 13 Q. And why did he suggest Cutter to you?
- 14 A. I was interested in getting to the West Coast.
- 15 Q. Good for you. When you joined, it was called  
16 Cutter at that time, I guess, wasn't it?
- 17 A. Right.
- 18 Q. And when you joined Cutter, were you working under  
19 the direction of -- what was it -- Dr. Cabasso who  
20 was head of research and development?
- 21 A. Ultimately it was Dr. Victor Cabasso. My initial  
22 supervisor was John Lundblad.
- 23 Q. And was Dr. Mozen in the organization at that  
24 time?
- 25 A. Yes.

- 1 Q. What department did you work in?
- 2 A. I think it was known as the Department of
- 3 Biochemical Development.
- 4 Q. And was your role primarily at that time working
- 5 on new products, or improvements to existing
- 6 products?
- 7 A. I think my initial assignment really was to look
- 8 at existing methods and primarily looking at new
- 9 equipment and to look at ways to improve the
- 10 current processes.
- 11 Q. What was John Lundblad's title, do you know?
- 12 A. I think he was manager of Biochemical Development.
- 13 Q. All right. And as there work in your department
- 14 going on with Factor VIII and Factor IX at the
- 15 time you joined Cutter?
- 16 A. I believe there was.
- 17 Q. And who was primarily doing that work?
- 18 A. It was being done by Dr. George Mitra and Kathryn
- 19 Fillmore.
- 20 Q. All right. We were talking about the hepatitis
- 21 risk before, and I don't want to dwell on this
- 22 because we have already covered it in part, but
- 23 did you also wear special clothing when you were
- 24 working with these plasma products?
- 25 A. Not any more than I would as part of general

- 1           laboratory practice. You wear a laboratory coat.  
2           When I was working with plasma, I'd wear gloves,  
3           but that was about the level.
- 4       Q.   Did you wear a mask?
- 5       A.   Off and on, depending on how I saw the issue I was  
6           dealing with.
- 7       Q.   Right. Now, is it fair to say that you, up until  
8           the time you joined Cutter, you had not worked in  
9           the research end with respect to any of the plasma  
10          proteins?
- 11      A.   That's fair.
- 12      Q.   All right. And just looking at your CV covering  
13          the entire period of time you were at Cutter, did  
14          you ever publish any article in a peer review  
15          journal having to do with coagulation proteins?
- 16      A.   No.
- 17      Q.   Have you ever, even up until the present time?
- 18      A.   No.
- 19      Q.   It's my understanding you don't presently work  
20          with plasma proteins. Is that right?
- 21      A.   Yes.
- 22      Q.   During the time period that you were at Cutter --  
23          which is, it looks like, June of 1976 to April of  
24          1980. Is that right?
- 25      A.   That's right.

- 1 Q. Were you in the same department the entire time?
- 2 A. Yes, I was.
- 3 Q. And during the entire time, was your supervisor
- 4 Dr. Lundblad?
- 5 A. Yes.
- 6 Q. During the period June of 1976 to April of 1980,
- 7 did you belong to any of the professional
- 8 organizations which had as their principal or
- 9 primary focus coagulation proteins?
- 10 A. No, I didn't belong to any professional society.
- 11 But as part of attending meetings at conferences,
- 12 I was exposed to some of the work that was going
- 13 on.
- 14 Q. Well, my question right now just had to do with
- 15 the organizations themselves. Were you a member?
- 16 A. No.
- 17 Q. Did you submit any articles for peer review that
- 18 were not accepted having to do with the
- 19 coagulation proteins?
- 20 A. No.
- 21 Q. Did you ever attempt to author an article having
- 22 to do with coagulation proteins?
- 23 A. No.
- 24 Q. When you say meetings you attended, what meetings
- 25 are you talking about?

- 1 A. There were some biological meetings that were  
2 held. I'm not too sure specifically about the  
3 specific names of those meetings, but they were a  
4 mix of biological sciences and engineering, and  
5 sometimes looking at applications. I don't  
6 consider myself a basic research person.
- 7 Q. I understand. Did you ever make any presentations  
8 at any of those meetings having to do with Factor  
9 VIII or Factor IX?
- 10 A. No.
- 11 Q. Current to the time you -- that is, as of the time  
12 you joined Cutter, had you ever performed yourself  
13 a Factor VIII or a Factor IX assay?
- 14 A. No.
- 15 Q. There came a time period, in my recollection, in  
16 October of 1978 when you -- for reasons which we  
17 will discuss in more detail, I just want to get a  
18 time frame for the moment -- when you began to  
19 explore the possibility of stabilizing gamma  
20 globulins. Is that correct?
- 21 A. Yes.
- 22 Q. All right. Can you just give me an overview, and  
23 if I have any questions about it, the details of  
24 it, I will ask you. But give me an overview of  
25 what you did between the time you joined Cutter in

1 May of 1976 up until October of 1978.

2 A. Yes. Well, I think for the first six or seven  
3 months of 1976, my major aspects of work dealt  
4 with looking at new equipment, specifically the  
5 separations type of equipment, ultrafiltration,  
6 diafiltration equipment, again looking at the ways  
7 trying to improve the manufacturing process.

8 I also got involved in getting -- doing  
9 things similar to what I was doing at Armour,  
10 looking at the entire Cohn fractionation process,  
11 again the albumin, gamma globulin component,  
12 looking at ways to improve the efficiencies, again  
13 very similar to what I had started off at Armour.  
14 But that was what was going on during 1976.

15 Q. What products -- you said ultrafiltration,  
16 diafiltration, new equipment. What products  
17 specifically?

18 A. Primarily 5% albumin, 25 albumin, 69.5% gamma  
19 globulin.

20 Q. Right. You were working with the products  
21 manufactured from Cohn fractionation, then that  
22 tells me, and not specifically Factor VIII. Is  
23 that right?

24 A. That's right.

25 Q. Okay. After the first six months, what kind of

1 work were you doing?

2 A. After the first six months, I actually began to  
3 get involved in a new product that Cutter was  
4 working on known as a Modified Immune Serum  
5 Globulin, and I will call it MISG, because it's a  
6 lot easier. There was this new product that  
7 Cutter was working on, and the issues they were  
8 facing was that they had completed much of the  
9 research work that was involved, and it involved  
10 adding various chemicals to the Modified Immune  
11 Serum Globulin, and they had to remove some of the  
12 residue components that they had added dealing  
13 with diafiltration, removal of components. And I  
14 got involved in terms of optimizing that process,  
15 running it at a larger pilot scaling, and  
16 beginning to supply material for clinical trials.  
17 That began towards the end of '76 and moving into  
18 1977.

19 Q. '77?

20 A. Yes, 1977. A number of issues connected with  
21 general aspect of scaling up. For example, very  
22 often not all of the reagents would be removed as  
23 rapidly as they should be removed, to worry about  
24 it, to the conditions on looking at  
25 reproducibility; some of the general standard

1 aspects that one looks at in terms of  
2 manufacturing some of these proteins.

3 Clearly as part of my role in this being a  
4 new immune serum globulin, one of the aspects was  
5 how do you formulate this new Modified Immune  
6 Serum Globulin, and that was my entree into  
7 looking at formulation and stabilization, but it  
8 was a blend of getting away from manufacturing  
9 into some of the scientific areas.

10 While that was going on, towards the middle  
11 of 1977, I was informed by Dr. Cabasso, but  
12 through Milt Mozen and John Lundblad, the need to  
13 switch to a freeze-dried formulation of MISG. So  
14 I began to look at ways to get a freeze-dried  
15 material, and again started a process of looking  
16 at excipients and stabilizers to freeze dry. And  
17 that went on towards the end of 1977 into the  
18 early part of 1978.

19 Q. 1978?

20 A. Yes, 1978.

21 Q. May I stop you there for just a moment?

22 A. Yes.

23 Q. First of all, we both understand this but so the  
24 Jury does, the gamma globulin product that we're  
25 talking about is not a coagulation protein. Is

- 1           that correct?
- 2       A.    That's correct.
- 3       Q.    What kind of gamma globulin product were you
- 4           working with? Was it for a specific virus or was
- 5           it for general purposes?
- 6       A.    It was basically for general purposes.
- 7       Q.    And were you working on a manufacturing process
- 8           that differed from the standard Cohn fractionation
- 9           of gamma globulin?
- 10      A.    It was an extension of the Cohn process.
- 11           Actually, our feed material was Fraction II, which
- 12           was the end of the Cohn process, and we took that
- 13           material as raw material and then went on to
- 14           develop a process.
- 15      Q.    Initially, was it the intent of the researchers at
- 16           Cutter to develop this product in a way that would
- 17           allow it to be administered intravenously?
- 18      A.    Yes, I think that was a very specific aim, to
- 19           develop an intravenously acceptable gamma
- 20           globulin.
- 21      Q.    Did that change when the decision was made to try
- 22           to develop a lyophilized product?
- 23      A.    No.
- 24      Q.    It was still intended to be administered --
- 25      A.    It was still intended, but we were looking at --

1           there were -- in terms of exporting the material  
2           to countries that may not have adequate  
3           refrigeration, there was a need, apparently, to  
4           look at a lyophilizing the product.

5       Q.   Now, you mentioned that as part of working out the  
6           biochemical engineering of lyophilizing this  
7           product, let's say, you began to think about and  
8           do experiments with stabilizing MISG. Is that  
9           correct?

10      A.   Yes, that is correct.

11      Q.   All right. Is this the first work that you had  
12           done up to that point stabilizing a protein?

13      A.   Beyond my experience at Rutgers, this was the  
14           first application looking at methods of  
15           stabilization.

16      Q.   All right. Well, specifically, it differed  
17           significantly from the work you did at Rutgers,  
18           didn't it?

19      Q.   It differed in the sense that it's immobilization  
20           on a different matrix, but I think the concepts of  
21           stabilization and the concepts, I think -- yes, it  
22           was my first experimental plan, but I was working  
23           -- I felt I had a good idea.

24      Q.   No, I understand. You drew from your experiences  
25           in stabilizing noncoagulation or nonplasma

1 proteins in the work that you did trying to  
2 stabilize a plasma protein. Is that right?

3 A. (no audible response)

4 Q. Is that right, sir?

5 A. Yes.

6 Q. Okay. Fair enough. So how did you familiarize  
7 yourself with the field of protein stabilization  
8 in preparation to design the experiments and to do  
9 the work trying to stabilize MISG?

10 A. If you don't have --

11

12 MR. JACKSON: Hang on.

13 MR. PRICE: I'm sorry.

14 MR. JACKSON: I'm sorry. There's an  
15 objection.

16

17 DEPOSITION OF DR. FERNANDES RESUMED

18 Q. If you didn't have to do --

19

20 MR. GOODELL: Where are you?

21 MR. JACKSON: I'm on line 14 of page 43.

22 There's a foundational objection and Mr.

23 Spivey just continued, so I'll just continue.

24 MR. GOODELL: Thank you.

25

1       **DEPOSITION OF DR. FERNANDES RESUMED**

2       Q.   If you didn't have to do any research and you  
3            didn't have to explore the scientific literature  
4            in any way, if you drew on your own general  
5            knowledge of protein stabilization when you began  
6            your work on MISG, please say so.

7       A.   There are a couple of aspects, yes. One was just  
8            my general knowledge, but I also looked -- we were  
9            also looking at other products. There were other  
10           gamma globulin products that were available not in  
11           the U.S. but in other countries, and my first  
12           attempt was to look at some of the excipients that  
13           they had been using and to begin.

14           Because, looking at gamma globulin, we did  
15           have an intramuscular product which was 16.5%, and  
16           it had glycine and sodium chloride in it. My  
17           first indication, well, let me work around those  
18           particular excipients, look at what other  
19           companies had commercial products available, look  
20           at what they were using and begin to formulate a  
21           product around that.

22

23                   **MR. JACKSON:** A plan around that?

24                   **MR. PRICE:** I'm sorry, formulate a plan  
25           around that.

1       **DEPOSITION OF DR. FERNANDES RESUMED**

2       Q.   Did you understand at the time that the excipients  
3           that you mentioned in the previous, the older  
4           generation gamma globulin product, were in the  
5           product for purposes of stabilization?

6       A.   I assumed that they were there for a reason, and  
7           the reason could have been either for  
8           stabilization or to maintain solubility of the  
9           material at that particular concentration.

10      Q.   Well, were you aware that the chemicals that you  
11           identified had been reported in the literature as  
12           being general stabilizers for some proteins  
13           current to 1976 and 1977?

14      A.   I don't know whether they may or may not have been  
15           referred to.

16      Q.   So basically, if I understand what you are saying,  
17           your initial selection of stabilizers to try to  
18           work with really came from your gaining knowledge  
19           of what was being used in other manufacturers'  
20           products and what had been used previously by  
21           Cutter in their older generation products. Is  
22           that correct, sir?

23      A.   Yes.

24      Q.   All right. And what stabilizers did you begin  
25           working with and when did you begin that work?

1       A.   Well, I think some of the initial work began in  
2           the early part of 1977, and I did achieve success,  
3           at least I felt I had achieved success at that  
4           time within the constraints of looking at sodium  
5           chloride, and I mentioned the other one was the  
6           glycine. Within those two, working on a small  
7           scale and an essential test where you freeze-dry  
8           the material, you reconstitute it, you look at it  
9           in terms of its visual clarity.

10               But also because gamma globulin was a pure  
11           protein, I had the opportunity to take it through  
12           some functional tests, run the standard analysis  
13           that one would do, which was extremely helpful to  
14           try to get me to understand that I was achieving  
15           some level of success. So that moved fairly  
16           rapidly.

17       Q.   Now, when you say visual clarity, I know what you  
18           mean, but again let's be sure the Jury does. If  
19           the material is cloudy when reconstituted that's  
20           an indication, perhaps, that you've altered the  
21           gamma globulin protein. Is that correct?

22       A.   It would be the ultimate result on a cascade of  
23           processes, and ultimately presumably it would come  
24           out of solution.

25       Q.   So clarity is a way of at least visually

- 1           confirming that perhaps you have been able to  
2           stabilize the gamma globulin protein during the  
3           lyophilization process. Is that right, sir?
- 4       A.   Not completely. I think it offers you the first  
5           step as a general way in your preliminary analysis  
6           to say yes, it looks like it's remaining in  
7           solution. That doesn't necessarily mean that if  
8           one put it to an assay or a test, because this  
9           full concept of stabilization occurs in various  
10          levels.
- 11       Q.   I understand.
- 12       A.   At the very gross, first level, yes.
- 13       Q.   In other words, if it was cloudy that would be  
14           good news; if it was clear, it would --
- 15       A.   It would require further investigation.
- 16       Q.   Let's do further investigation, yes. What  
17           happened to MISG during the lyophilization process  
18           if stabilizers weren't added?
- 19       A.   Several things could presumably happen. One, in  
20           which the material in the vial would basically  
21           collapse. One of the things one wants to achieve  
22           in a freeze-dried preparation is to make sure that  
23           if you have it filled at a ml fill, you expect the  
24           freeze-dried cake to be one ml. If you get the  
25           cake completely collapsing on you, it would be an

1           indication that the lyophilization was not  
2           working. In some instances with some excipients  
3           the cake would completely collapse. That would be  
4           one method.

5           The other method would be the reconstitution  
6           time; and the third would be a change in some of  
7           the activities. We were measuring the purpose of  
8           running -- we had, again, dealing with MISG, the  
9           ability to measure the anti-complement activity.  
10          We knew the general nature of ISG gamma globulin.  
11          We could calculate the amount of high molecular  
12          weight, the heavy and light chain, to get some  
13          assessment, but that would be the general scenario  
14          we'd make.

15        Q. But I take it from your explanation that one of  
16           the things you were concerned about was that if  
17           you didn't add stabilizers to the MISG that some  
18           element of the protein could be inactivated or  
19           denatured during the lyophilization process. Is  
20           that correct, sir?

21        A. Yes, we felt we were adding it for a reason.

22        Q. Now, you mentioned this work, and I think I  
23           interrupted an answer of yours, and I apologize.  
24           You mentioned that this work was occurring in the  
25           beginning of 1977 and that you thought at the

1 bench scale that you had succeeded in coming up  
2 with a material that looked like it has been  
3 stabilized during the lyophilization process.  
4 Will you please pick up from there, sir?

5 A. One of the aspects that we would work on was once  
6 we lyophilized a material we would hold it for  
7 certain periods of time. That is generally known  
8 as an accelerated stability test. We'd have some  
9 vials at 5°, some at room temperature, and some at  
10 an elevated temperature, usually 37°, and so it  
11 holds these vials from four to five weeks. So  
12 through this analysis we make an assessment.

13 Q. By the way, were you performing the assays on MISG  
14 yourself or was some other department in the  
15 company doing that work for you?

16 A. At that time, we were working in a fairly large  
17 team. My role in it was primarily the  
18 manufacturing aspects, the lyophilization, but I  
19 depended very heavily on the analytical group.

20 I'm not an analytical man. I would give them  
21 samples. I would say, "Please measure the levels  
22 of anti-complement activity." I made the  
23 assumption that the analytical people were  
24 reliable; I mean the team. But my response was,  
25 "These are the results and I'm going to use them,

1 the best you have available."

2 Q. No, I'm not questioning that. But I think the  
3 point you are making is that the assay work was  
4 done --

5 A. All the assay work was done by -- except the  
6 reconstitution time, I could do that.

7 Q. Incidentally, were you able to design these  
8 experiments yourself?

9 A. Yes, I was doing the design of the lyophilization.

10 Q. Okay. I don't mean to keep interrupting you, but  
11 continue with the description of the work you were  
12 doing with MISG in 1977.

13 A. In addition to the work on lyophilization, which I  
14 had started somewhat during the March-April period  
15 of 1977, I was also dealing with other  
16 manufacturing problems with MISG that took me  
17 through most of the rest of the year essentially.  
18 Towards the end of 1977, we felt we were ready to  
19 begin manufacturing clinical batches of material,  
20 and I think in late 1977 or the early part of  
21 1978, we began the manufacturing process. And --

22 Q. Let me just stop you there. There is a patent,  
23 which is listed on your CV, that has a date of  
24 1980 attached to it, which is entitled, quote,  
25 "Stabilization (Formulation) of Intravenous Gamma

1 Globulin". When did you file for that patent?

2 A. It was probably around the middle of 1978.

3 Q. All right. And what stabilizers did you identify  
4 in the patent, this 1980 patent?

5 A. I think we looked at a variety of carbohydrates.  
6 Could have been sucrose, mannitol, dextrose. I  
7 don't recollect specifically beyond that. And  
8 maltose.

9 Q. When did you begin working with these stabilizers?

10 A. I began working with these stabilizers in April of  
11 1978.

12 Q. And again, before you selected those stabilizers  
13 to do your experiments, did you do any research in  
14 the protein biochemistry field to see whether, in  
15 fact, those stabilizers had been described as  
16 having stabilizing aspects with other types of  
17 proteins?

18 A. No, I didn't see a particular need in this case to  
19 get involved in those aspects. What happened was  
20 that while I began running some of these  
21 manufacturing batches, after a few batches we  
22 realized that material was, indeed, coming out of  
23 solution. My initial assumptions of stabilizers  
24 that I generated in 1977 were not working, and  
25 there clearly was a concern within the

1 organization that we need to do something very  
2 rapidly to solve this problem.

3 Again at that time there was an in-process  
4 assay that was used in manufacturing which  
5 involved heating gamma globulin to 57° for four  
6 hours, and the manufacturing operators would look  
7 at it and say, "Did it gel or did it not gel?",  
8 and that was a nice test. And so I felt rather  
9 than waiting for four to six weeks and keeping  
10 material at refrigeration conditions, I would look  
11 at this shot quick test.

12 Again, having recognized that there were  
13 other competing gamma globulin products around,  
14 one of them was intraglobulin, which had low  
15 levels of dextrose in it, I think 2.5% percent  
16 dextrose in it, whereas I didn't look at those in  
17 1977 because I felt I had already solved this  
18 problem with sodium chloride and glycine. I  
19 started looking at dextrose, and then I think  
20 quite surprisingly, as far as I was concerned, we  
21 saw a significant change when we put, when we  
22 formulated MISG in 5% dextrose when subjected to  
23 this accelerated heat stability test.

24 And once I saw it work with 2.5% glucose, at  
25 least we were seeing an effect, I began to

1 explore, you know, the best types of sugars to be  
2 made available, and I spent most of 1978, between  
3 then till October sort of researching this  
4 particular concept.

5 Q. I understand. Let me just be sure I have this  
6 clear, though. Your selection of the carbohydrate  
7 dextrose came not from a review of the protein  
8 biochemistry literature but simply from your  
9 knowledge that there was another competitor's  
10 product on the market who used a certain  
11 concentration of dextrose in their manufacturing  
12 process. Is that correct, sir?

13 A. That was essentially. I did not go into research  
14 at any level of detail. My first reaction was to  
15 see what's available, can I modify something that  
16 apparently people have used.

17 Q. Right. And if I understand the way you were able  
18 to quickly learn that MISG was becoming  
19 destabilized during the manufacturing process was  
20 when this quick test, this 57° centigrade for four  
21 hour test, you were seeing the MISG coming out of  
22 solution. Is that right?

23 A. In terms of the manufacturing operation, what got  
24 me going was that on one particular manufacturing  
25 run, material started coming out of solution

- 1           during the act of filling.
- 2       Q.    Indicating that --
- 3       A.    Indicating that there was a severe instability
- 4           going on.  It is then that I realized that there
- 5           is this quick test that I could use to sort of
- 6           measure in very, very -- now this was a method to
- 7           identify in very, very -- this is in gross terms,
- 8           the ability of protein gamma globulin, in this
- 9           case, to come out of solution.
- 10      Q.    So getting back again to your patent, you believe
- 11           that the patent which was granted in 1980 and
- 12           filed for, I think you said sometime in the spring
- 13           of 1978 -- didn't you say the patent was filed,
- 14           did you say '77 or '78?
- 15      A.    I said I don't recollect specifically, but I know
- 16           the work occurred during the period of 1978, and
- 17           sometime during that we may have filed a terms of
- 18           disclosure.
- 19      Q.    I think you might have said mid '78, but that's
- 20           okay.  Of course, we are going to get a copy of
- 21           the patent.  But in the patent, did you identify
- 22           various carbohydrates that you believed at varying
- 23           concentrations could stabilize MISG or gamma
- 24           globulins, generally?
- 25      A.    Yes, I indicated that there was a range of

1           carbohydrate concentrations that would work for  
2           patent purposes and a range of carbohydrates, yes.

3       Q.   And was that -- and was the invention limited to  
4           intravenous gamma globulins?

5       A.   That one was limited specifically to intravenous  
6           gamma globulin.

7       Q.   Right. And in the patent, I assume, you had to  
8           cite prior art. Is that correct?

9       A.   One of the -- yes.

10      Q.   And who was responsible for identifying the prior  
11           art? Is that something you did yourself or did  
12           you have others at Cutter do the research, or  
13           what?

14      A.   I would do some of it, but essentially the patent  
15           attorney at Cutter would also do a more general  
16           wider search in a more formal way.

17      Q.   Sure. Well, let me ask you this much: Is it a  
18           correct statement that your first kind of look  
19           into the research or into the literature, the  
20           scientific literature, having to do with protein  
21           stabilization in the plasma context was when you  
22           were researching the prior art for purposes of  
23           filing this patent?

24      A.   On this particular case in dealing with the  
25           dextrose on the MISG patent, I didn't carry out

- 1           any significant literature search at that point.  
2           It came up, I made a recommendation that I thought  
3           this seemed to work very well, and moved on.
- 4       Q.    I understand. I was asking you though, you said  
5           you had done some research, and others at Cutter  
6           did other research.
- 7       A.    Yeah.
- 8       Q.    But was that research that you did for purposes of  
9           helping to identify prior art the first glimpse  
10          you had into the literature of protein  
11          stabilization?
- 12      A.    Yes. It was more sort of a validation or  
13          confirmatory finding, here we had got something  
14          that I felt worked. We had done a few experiments  
15          and it looked like it worked. The next step was  
16          talking about how one would apply it. Yes, you  
17          need to look at the literature. You need to move  
18          on. So it was kind of a confirmatory exposure  
19          more than anything else.
- 20      Q.    Do you recall as you sit here today any of the  
21          prior art that is cited in the 1980 patent?
- 22      A.    You know, it's hard for me. It's 18 years.
- 23      Q.    Sure.
- 24      A.    I don't think I can track back.
- 25      Q.    That's okay. Do you know, for example, whether

1           you cited the work of Seegers in the 1980 patent?

2       A.    I don't. I don't recollect.

3       Q.    Well, that's fine. I don't blame you, and we can  
4           certainly look in the patent, obviously.

5       A.    Right.

6       Q.    I take it from a previous answer that you gave  
7           that Cutter had the ability to do patent searches  
8           and literature searches in terms of helping you  
9           identify prior art current to the 1977, '78 time  
10          frame. Is that right, sir?

11      A.    I think so. I think they did.

12      Q.    And who, other than yourself, is named as the  
13          inventor on the 1980 patent?

14      A.    I think it's Mr. John Lundblad and Dr. Will  
15          Warner.

16      Q.    I kind of understand how this works, I think. Dr.  
17          Lundblad was named as a co-inventor mostly because  
18          he was your supervisor at the time. Is that  
19          right, sir?

20      A.    Oh, no, not necessarily. I used to meet with John  
21          Lundblad at weekly meetings, we used to discuss  
22          some of these issues that came up, and I saw it as  
23          a joint relationship.

24

25                   MR. JACKSON: Inventorship.

1                    MR. PRICE: Inventorship. I'm sorry.

2

3                    **DEPOSITION OF DR. FERNANDES RESUMED**

4                    Q.    But is it correct that most of the hands-on work  
5                    was done by you?

6                    A.    Yeah, you know, science is a mix of two things. I  
7                    think there is somebody who does the work; there  
8                    is also the thinking process that goes on. So  
9                    yes, in this case I physically did it, but the  
10                   knowledge, the ability, the thinking process is a  
11                   joint process.

12                   Q.    I'm not suggesting --

13                   A.    I just want to make it clear.

14                   Q.    The hands-on work is done by you. Right?

15                   A.    Right.

16                   Q.    And I think you told us you designed most of the  
17                   experiments. Is that correct?

18                   A.    Yes.

19                   Q.    Now, continuing through the year 1978, it's  
20                   obvious to me from looking at the materials that a  
21                   question came up at some point in the process as  
22                   to whether this product, this MISG product, would  
23                   be virally safe when administered to patients. Is  
24                   that true?

25                   A.    Not to my knowledge. I don't recollect that issue

1 coming up.

2 Q. You don't recollect concern at Cutter that a  
3 change in the manufacturing process of gamma  
4 globulin could change the viral safety of the  
5 product?

6 A. I think I need to make it clear that MISG, the raw  
7 material for the MISG was really the last step of  
8 the Cohn process, so it was already known for  
9 quite some time that Fraction II material derived  
10 from the Cohn process was essentially free of  
11 hepatitis.

12 So we were using that as the raw material  
13 going forward. So I think from that standpoint I  
14 don't think there was a concern expressed.

15 Q. All right. Well, again, we can look at some  
16 documents, because you may decide later that you  
17 are in error on that. In October of 1978, there  
18 was a disclosure filed in the company which you  
19 authored. Is that true?

20 A. I'm not sure which particular disclosure you are  
21 referring to.

22 Q. You know what, I was only able to get my hands,  
23 for some reason, on one copy of this. Can we take  
24 a minute and get a copy?

25 You wanted to make a clarification. I think

1 I understand what that clarification was. We had  
2 been talking up to that point about MISG.

3 A. Which had its own patent application.

4 Q. I understand. And this really has to do with  
5 something completely different, doesn't it?

6 A. Yes, it does.

7 Q. So we've been kind of on a time line, and we were  
8 talking about, let's say, the first half of 1978  
9 when you were working with the MISG product and  
10 stabilization with carbohydrates. When did your  
11 emphasis change from MISG to other products?

12 A. As I said, during the early part of 1977 we were  
13 having a problem with the instability of MISG, we  
14 had moved into looking at carbohydrates. We were  
15 also looking at the accelerated assay to 57° for  
16 four hours.

17 While that work was going on, I was also  
18 spending a lot of time in terms of manufacturing  
19 issues of scale-up, and every day we would be  
20 running, you know, you'd take a sample, put it in  
21 a hot water bath, go and do stuff, come back in  
22 four hours and assay this material. It was  
23 working pretty well.

24 But I think during August, or sometime during  
25 the end of summer, I probably was not being as

1 careful as I should have been because I left these  
2 samples in for a lot longer than I had planned to.  
3 It's not at all clear looking back now. I  
4 actually know it happened. If you tell me  
5 precisely on which day it happened, it was an  
6 accident and these things happen, left it  
7 overnight.

8 I suspected that when I came back in in the  
9 morning, I was first of all quite surprised to see  
10 these things in the hot water bath and said,  
11 looked at it, yeah, it was opalescent, but it was,  
12 I was thinking mentally of the stuff coming  
13 totally out of solution. So surprising in my  
14 mind, I said, well, I've got to figure out what's  
15 going on here.

16

17 **MR. PRICE:** Your Honor, might I ask that  
18 this be an appropriate time to take a break?  
19 I'm getting very hoarse.

20 **THE COURT:** Yeah, that's fine. I  
21 understand that. We're going to take a brief  
22 break while the witness regains his voice.

23

24 **WHEREUPON THE JURY WAS EXCUSED FROM THE**  
25 **COURTROOM**

1 COURT WAS RECESSED

2 COURT WAS RECONVENED

3

4 THE COURT: Okay. This is on the  
5 objection of Fernandes, and it's on what now?

6 MR. GOODELL: Eighty-six, line 16.

7 THE COURT: Eighty-six, line 16. Okay.

8 (Court reviews deposition)

9 THE COURT: Okay.

10 MR. GOODELL: Really, it's just what it  
11 says. The objection is it's unintelligible  
12 and it's improper, and that's the only  
13 objection I've got in this.

14 MR. JACKSON: We want to ask that  
15 question, Judge.

16 THE COURT: The objection's going to be  
17 overruled. I can follow that.

18 All right.

19

20 WHEREUPON THE JURY WAS RETURNED TO THE  
21 COURTROOM AND SEATED IN THE JURY BOX

22

23 THE COURT: Okay.

24

25 DEPOSITION OF DR. FERNANDES RESUMED

1 Q. Let me just stop you here for a second. What time  
2 period are we talking about?

3 A. This is mid to late summer. July, August,  
4 probably closer to August, of 1978.

5 Q. Okay. This discovery or surprise, or however you  
6 want to characterize it, the product that you were  
7 working with at the time was MISG?

8 A. Yes, all MISG.

9 Q. Right. And still with the same stabilizers we are  
10 talking about, carbohydrates?

11 A. At this time, we had moved closer to looking at  
12 maltose, and specifically looking at maltose at 5%  
13 to 10% primarily for osmolarity issues, other  
14 issues, but we were also trying to find a carb  
15 that is pharmaceutically acceptable.

16 It turned out at this particular case,  
17 researching out the particular types of sugars,  
18 maltose seemed a nice one for a couple of reasons,  
19 because Cutter at one point was also making some  
20 intravenous drips and they had a source of  
21 maltose. This source was a company in the Far  
22 East known as Otsuka, but we felt that this was  
23 the best quality maltose, so we were moving in  
24 that particular direction. Yes, so it was maltose  
25 at that particular time.

- 1 Q. Had you convinced yourself, let's say by  
2 July-August of 1978 that you had successfully  
3 stabilized MISG with a concentration of maltose?
- 4 A. It was becoming more and more clear to me, and I  
5 think this was solving our problems.
- 6 Q. Now, were you doing the assay work yourself? As I  
7 understand the process, you were doing this heat  
8 experiment or heat situation where you were  
9 heating the material up to 57° centigrade for four  
10 hours, then you were doing an assay to see whether  
11 you had a decent level of activity. Is that  
12 correct?
- 13 A. Yes, but we weren't doing it at each and every  
14 experiment. I was doing a couple and move on.
- 15 Q. Were you doing the assay yourself?
- 16 A. No. In fact, I wasn't doing any assays as far as  
17 that project was concerned personally.
- 18 Q. As we discussed before, you handed them off to the  
19 analytical department, they did the assays and  
20 reported back to you. Is that correct?
- 21 A. Yes.
- 22 Q. Did you do any intentional experiments to learn  
23 how much beyond 57° centigrade or how much time  
24 beyond four hours you could heat this product and  
25 still keep it in solution?

1       A.   No. I wasn't motivated. I was using this just as  
2           a test, you know. The concept in my mind was we  
3           need to keep this material stable at refrigeration  
4           conditions. That's where we were heading. It was  
5           a quickie test to help me do it.

6                 So that wasn't the motivation until this  
7           incident occurred, and that's when I think -- you  
8           know, these things happen sometimes. There was  
9           something here that I need to investigate, because  
10          this is an event that I did not expect.

11       Q.   Right. You would have expected, going into it,  
12           that if you heat the material overnight for many,  
13           many, many hours -- how many hours was it, by the  
14           way, do you think?

15       A.   I don't know. Sometime 3:00 or 4:00 in the  
16           afternoon. I was planning on coming at 7:00 to do  
17           it. I probably came in the next day at 7:30,  
18           8:00. So fifteen, twenty hours.

19       Q.   Right. And by physical observation, you saw that,  
20           gee whiz, some of this stuff is still in solution,  
21           it's not completely destabilized. Correct?

22       A.   It's not completely destabilized.

23       Q.   Now, when you were reviewing materials in  
24           preparation for this deposition, did you identify  
25           in the lab notebooks for the year 1978 a day or

- 1           two when this discovery probably occurred?
- 2       A.   Looking through my notebooks, I did not  
3           specifically note this particular incident, but I  
4           remember the moment it happened. Within a few  
5           days after in talking to John we said, "Let's do  
6           an experiment to confirm this finding. Let's  
7           develop a system to do it now that it looks like  
8           we've got a handle here." So I do have, and that  
9           information is recorded.
- 10      Q.   Can you pull out the experiments that resulted  
11           from this overnight discovery that you made so  
12           that we can look at them and mark them as  
13           exhibits, please?
- 14      A.   I have got to remind myself for mid '78. I think  
15           if you look at lab notebook 2021, page 25, I  
16           believe that's the start of this controlled  
17           experiment that I did.
- 18      Q.   All right. The thermal stability. All right.
- 19      A.   And what you see on that page is the absorbance or  
20           the transmittance of light. I had a wavelength of  
21           580 nanometers for the first two columns. The  
22           second two columns indicate absorbance and  
23           transmittance after heating for four hours at 57°.
- 24      Q.   Let me just -- the record is going to get all  
25           confused if you explain this and I haven't asked

1           you a question, so let me just see if I can ask  
2           you some questions. First of all, the page of the  
3           lab notebook we're referring to, which has a  
4           number 25 at the top right-hand corner, and it's  
5           lab book 2021, it has a date at the bottom of  
6           September 6, 1978, and it has your signature on  
7           it. Is that correct?

8       A.   Yes, it is.

9       Q.   We haven't really talked about lab notebooks up to  
10          this point, but it's my understanding that this is  
11          a way a company who performs bench research  
12          documents experiments performed on a day-to-day  
13          basis for lots of reasons, including potentially  
14          patent situations. Is that correct?

15      A.   It's a way to keep track of the work that one  
16          does.

17      Q.   Sure. So that if we look at this notebook, even  
18          though it's a whole bunch of years ago, you feel  
19          fairly confident, I assume, that on September 6,  
20          1978 these are the experiments that you were  
21          doing. Is that correct?

22      A.   That's what the record shows.

23      Q.   Right. And the notebook is actually filled out in  
24          your handwriting, isn't it?

25      A.   Yes, it is my handwriting.

- 1 Q. Can you confirm, as you have before, that these  
2 experiments were designed by you?
- 3 A. Yes.
- 4 Q. And again, the Jury is -- they are not scientists,  
5 most likely, and neither am I. Just give me in  
6 lay terms what you were trying to confirm in the  
7 experiments here.
- 8 A. Well, if you look on page 25 and page 26 and page  
9 27 --
- 10 Q. Right.
- 11 A. -- what I have done is to take several samples of  
12 MISG at varying levels of maltose, you can see the  
13 range going from 1% maltose to 15% maltose, heated  
14 these samples, different sets of samples, either  
15 for four hours, which is shown on page 25 --
- 16 Q. And four hours, that was the time --
- 17 A. At 57°.
- 18 Q. -- which was the --
- 19 A. The standard test.
- 20 Q. Which you had been doing before.
- 21 A. Correct.
- 22 Q. Okay. Go ahead.
- 23 A. On the next page, heated a second set of samples  
24 to eight hours at 57°, and going onto page 27,  
25 sixteen hours under Item 5. And I recollect now,

1 after going back and looking specifically at my  
2 lab notebook, that I did make an error. It's  
3 probably not 47° centigrade as in Item 5, it's  
4 probably 57.

5 But anyway, sixteen hours, and then another  
6 set at twenty-four hours. And I used this  
7 information to look at the change in transmittance  
8 of light at all of these conditions, and this data  
9 essentially confirmed the earlier finding that  
10 whereas one would expect MISG to come out of  
11 solution generally --

12 Q. Or to destabilize?

13 A. -- or to destabilize it, here this wasn't an  
14 answer. But it was sort of the first gut level  
15 feeling that this is potentially useful as a  
16 stabilizer.

17 Q. Right. Now, if I understand what you described,  
18 again just to put it in lay terms, you were doing  
19 work at a constant temperature in each of these  
20 kinds of experiments and varying the period of  
21 time. Is that right?

22 A. Varying the period of time, as well as looking at  
23 several concentrations of maltose.

24 Q. And those experiments, according to these pages,  
25 seem to have occurred on September 6th and

1 concluded, well, really over two days, the first  
2 day being September 6th, the second day being  
3 September 11th. Is that correct?

4 A. Yes, if that's what the record shows.

5 Q. Well, just look at the notebook pages. It seems  
6 to me that's what it shows.

7 A. Yes.

8 Q. All right. Okay. Let's mark these three pages of  
9 the laboratory notebook as the next exhibit.

10 Did you continue your experiments with MISG  
11 beyond the two days we've discussed so far,  
12 September 6th and September 11th?

13 A. For this particular experiment?

14 Q. Or other experiments where you were exploring this  
15 concept that maltose could extend the period of  
16 stabilization at 57° centigrade beyond four hours.  
17 Did you continue those experiments?

18 A. I actually continued working on the concept now  
19 which I haven't yet explained to you, but at this  
20 time I began to get the recognition that maybe  
21 this could be used as a method of pasteurization.  
22 So this was a change in my view, also.

23 There was subsequent experiments in which I  
24 began to try to explore this issue about  
25 pasteurization, again recognizing that these

1 experiments did not show complete clarity of the  
2 product but definitely lowering levels of  
3 opalescence.

4 Q. Can you explain the concept for me, please?

5 A. The concept was that I felt that heating something  
6 to 57° for greater than four hours allows one the  
7 possibility of pasteurization of gamma globulin.

8 Q. Okay. And again, we don't need to, I certainly  
9 don't want to unless you feel it's necessary, to  
10 go page by page through your laboratory notebooks.  
11 But let's, if we can, just summarize the work that  
12 you did between, say, September of 1978 up to the  
13 date of the disclosure, which we put in front of  
14 you right at the end of the last break.

15 A. The work that went on primarily after this stage  
16 was looking at pasteurization. I classified  
17 pasteurization now as 60° centigrade for some  
18 period of time.

19 Q. Were you focusing on 60° centigrade?

20 A. Focused in on 60° centigrade, because I did know  
21 that albumin was pasteurized at that condition. I  
22 was also aware of the fact that 60° is a minimum  
23 necessary condition. It may not be a sufficient  
24 condition, but it was one of the factors that said  
25 yes.

- 1 Q. So if I understand what you are saying, you felt  
2 that you had to get the temperature up to at least  
3 60° centigrade, and maybe more, in order to  
4 pasteurize any of these plasma proteins. Is that  
5 correct, sir?
- 6 A. At this point, yes.
- 7 Q. And so between September 11th of 1978 and October  
8 25th of 1978, just give me a general summary of  
9 the work you were doing.
- 10 A. What I did was to pasteurize MISG and begin to  
11 characterize the molecule as best I could, so I  
12 did a series of experiments looking at antibody  
13 titers, looking at ultracentrifugation, SDS page  
14 analysis, and all the analytical techniques that  
15 were available. Here we were dealing -- there was  
16 a lot of information on MISG at that point. That  
17 work sort of completed around October, and I used  
18 that as a basis for a patent disclosure.
- 19 Q. Right. Is that the patent disclosure that we have  
20 in front of you?
- 21 A. Yes, it is.
- 22 Q. All right. Now, did someone suggest to you that  
23 there would be an important benefit in terms of  
24 safety that would be added to MISG if it could be  
25 pasteurized?

1     A.   No.  In fact, that was something that came from  
2           me, and it -- I actually found myself trying to  
3           explain to several people why I felt it was  
4           important.  It wasn't something that came from the  
5           outside.

6     Q.   Right.  Why did you feel it was important?

7     A.   Well, one of the reasons I felt it was important  
8           is, again, my background from engineering coming  
9           into it, I was looking at various ways to improve  
10          processes.  This is my background and training and  
11          job to do it.

12    Q.   Sure.

13   A.   I did recognize that most of the manufacturing  
14          that was going on revolved around the Cohn  
15          manufacturing process that dealt with the use of  
16          ethanol and plasma, and there was a history of  
17          data that was available which said that material  
18          made by the Cohn process is essentially safe.  The  
19          albumin part of it is pasteurized.  The gamma  
20          globulin process historically is shown to be free  
21          of hepatitis.  But in the seventies I was  
22          beginning to become aware of new technologies that  
23          were becoming available.

24                 There was something like Farmacia, for  
25          example, --

1                   MR. GOODELL: There were companies.

2                   MR. JACKSON: Companies.

3                   MR. PRICE: There were companies. I'm  
4                   sorry. Push my glasses up.

5

6       **DEPOSITION OF DR. FERNANDES RESUMED**

7       A. There was companies like Farmacia, for example,  
8           that was talking about ion exchange, new methods,  
9           and there was a -- I won't say there was a  
10          concern, but people -- there was a feeling, "Look,  
11          if you are going to research all of these things,  
12          we've got to worry about what effect it's going to  
13          have on the manufacturing process," and I saw that  
14          as an out.

15                I said, "Look, if we find a way to pasteurize  
16          gamma globulin, we can take that off the  
17          headlines. Now we can explore other processes and  
18          at least not worry a lot about this." So that was  
19          my motivation going into it.

20       Q. Well, I think, if I understand what you are  
21          saying, you felt that pasteurization of MISG would  
22          add a margin of safety from a hepatitis standpoint  
23          at least, to the product. Is that correct, sir?  
24          Am I, sir? You were thinking about viral safety  
25          of this product, weren't you, sir?

- 1       A.    I actually saw it as an opportunity to allow us to  
2            explore wider manufacturing options.  Yes, part of  
3            it was.  I'm not too sure I zeroed in on it, but  
4            yes.
- 5       Q.    Well, tell me, if you can, any other reason you'd  
6            want to pasteurize MISG at 60° centigrade other  
7            than killing these viruses or potentially adding a  
8            margin of safety to the product?
- 9       A.    Well, that was a factor.
- 10      Q.    And if I understand what you said, and I think I  
11            do, this was an idea that you kind of had in your  
12            head.  This wasn't something somebody told you.  
13            This was something you came up with yourself.
- 14      A.    This particular case of pasteurization of MISG,  
15            this was also one in which I felt I had an answer.
- 16      Q.    Sure.
- 17      A.    I had solved a particular and I wanted to say,  
18            "Well, let's look at applications of this new idea  
19            that's come across."
- 20      Q.    And if I understand the way this went, I mean I  
21            have read all your stuff here, once you realized  
22            that MISG could be stabilized, you thought to  
23            yourself, "Well, I wonder if any of the other  
24            products of the Cohn fractionation can be  
25            stabilized and heated."  Is that right, sir?

1       A.   My belief generally from the history from the old  
2           Rutgers days was that proteins are generally not  
3           very heat stable.  So here I was beginning to get  
4           a sense that maybe there is an opportunity here to  
5           try and see where else I could put this  
6           information to good use, yes.

7       Q.   I understand.  So you actually started doing  
8           experiments on the other products made from the  
9           Cohn fractionation method.  Is that true?

10      A.   Yes, that's right.

11      Q.   And you began, it's obvious from the notebooks  
12           here, you began designing experiments that allowed  
13           you to do the same kind of work on albumin, for  
14           example, that you had done on MISG.  Is that  
15           right, sir?

16      A.   Yes.

17      Q.   And as your patent disclosure indicates, which we  
18           will mark as the next exhibit, one of your  
19           concerns was the opportunity to pasteurize these  
20           products to avoid the risk of transmitting  
21           hepatitis.  Is that true, sir?

22      A.   Yes.

23

24                   **MR. JACKSON:**  Okay.  The witness is  
25           presented with Exhibit 3, "Take a minute, if

1                   you don't mind, and read it. Then we don't  
2                   have to stop again and go through it."  
3                   Witness does.  
4

5       **DEPOSITION OF DR. FERNANDES RESUMED**

6       Q.   First of all, in Exhibit Number 3, a copy of which  
7            you have in front of you, this document was  
8            written in your own hand. Is that true, sir?  
9       A.   Yes, it was.  
10      Q.   And you are indicated at the top as being one of  
11            the inventors, together with Doctor -- is it  
12            Doctor or Mister?  
13      A.   It's Mr. Lundblad.  
14      Q.   What were his qualifications, by the way?  
15      A.   Mr. Lundblad, my personal knowledge was he had a  
16            wealth of experience in the fractionation  
17            industry, and he had been working there for I  
18            think thirty years when I joined.  
19      Q.   Sure. But he wasn't a Ph.D, obviously?  
20      A.   He wasn't a Ph.D, but he had a wealth of practical  
21            experience which we people in practical  
22            applications respected.  
23      Q.   Sure, I can understand that. Just give me a one  
24            or two sentence explanation of why you would fill  
25            out a document like this that we've marked as

1 Exhibit 3.

2

3 MR. GOODELL: Excuse me. Are you going  
4 to put this on the screen and introduce it,  
5 or not?

6 MR. JACKSON: No.

7 MR. GOODELL: Are you planning on doing  
8 others?

9 MR. JACKSON: Yes.

10 MR. BARR: Your Honor, --

11 MR. PRICE: Do you want it on the  
12 screen?

13 MR. GOODELL: Well, I'm thinking if  
14 you're going to use exhibits you ought to put  
15 them all on the screen and introduce them.

16 THE COURT: Well, I suppose if the  
17 witness were live and you handed him the  
18 exhibit you wouldn't necessarily have to put  
19 it on the screen. But, you know, I don't  
20 know what --

21 MR. JACKSON: These are just not  
22 exhibits we were going to introduce.

23 MR. GOODELL: I guess we should have  
24 done this in the beginning.

25 THE COURT: All right.

1                   MR. GOODELL: Perhaps it's my fault.

2                   But I think --

3                   MR. BARR: May we approach the bench?

4                   THE COURT: Yeah. I mean, the only  
5                   thing -- yeah, approach.

6

7                   THEREUPON COUNSEL APPROACHED THE BENCH  
8                   AND THERE WAS COLLOQUY OUT OF THE  
9                   HEARING OF THE JURY

10

11                  THE COURT: Okay.

12                  MR. JACKSON: I'm starting with line 20  
13                  on page 74.

14

15                  DEPOSITION OF DR. FERNANDES RESUMED

16                  Q. It's a document dated October 25, 1978, as we  
17                  said. Dr. Fernandes' name appears as one of the  
18                  inventors. It's entitled Pasteurization of  
19                  Albumin and Immune Serum Globulin in the Presence  
20                  of Carbohydrates. What's the purpose of a  
21                  document like this?

22

23                  MR. PRICE: May we take one moment  
24                  again, Your Honor?

25                  THE COURT: Okay.

1                   THEREUPON COUNSEL APPROACHED THE BENCH  
2                   AND THERE WAS COLLOQUY OUT OF THE  
3                   HEARING OF THE JURY

4  
5                   THE COURT: What was that one? It was  
6                   Deposition Exhibit Number 3. What number is  
7                   it going to be?

8                   MR. PRICE: Nine ninety-nine.

9                   THE COURT: Nine ninety-nine. And  
10                  that's his notebook?

11                  MR. JACKSON: It is his disclosure --

12                  THE COURT: Oh, yeah, that's right.  
13                  Okay.

14                  MR. JACKSON: -- dated October 25, 1978.

15                  THE COURT: Is there going to be an  
16                  objection now?

17                                 (laughter)

18                  MR. GOODELL: I don't have that much  
19                  nerve, Judge.

20                  THE COURT: Okay, fine. Then 999 will  
21                  come in.

22                  MR. JACKSON: Okay. On line 20, page  
23                  74.

24

25                  DEPOSITION OF DR. FERNANDES RESUMED

- 1 Q. It's a document dated October 25, 1978, as we  
2 said. Dr. Fernandes' name appears as one of the  
3 inventors. It's entitled Pasteurization of  
4 Albumin and Immune Serum Globulin in the Presence  
5 of Carbohydrates. What's the purpose of a  
6 document like this?
- 7 A. One of the things that we were asked to do at  
8 Chiron -- I'm sorry -- at Cutter was that whenever  
9 we came up with ideas that looked like they  
10 were potentially novel, because at this point we're  
11 not too sure about the novelty of it, it was  
12 important that we have it documented and sent to,  
13 I think, patent counsel. And they would put it in  
14 their books, document it, make sure they had a  
15 very specific date of generation of this idea.  
16 That was the purpose of it.
- 17 Q. Right. This kind of a document is important for  
18 patent purposes in order to date the invention.  
19 Is that right?
- 20 A. Yes.
- 21 Q. In case a dispute arises later as to who had the  
22 invention first. Right?
- 23 A. Essentially, yes.
- 24 Q. Now, with respect to the immune globulins and  
25 albumin that is identified in this disclosure, is

1           it correct that the purpose disclosed for  
2           pasteurizing the product at 60° centigrade has to  
3           do with the potential risk of hepatitis  
4           transmissibility through those particular Cohn  
5           fractions?

6       A.   Yes, in a sense, but I need to clarify it, and I  
7           was coming into this field as an engineering  
8           background. I always look at it in my personal  
9           terms. Yes, those are the words I did express and  
10          write down. Ultimately as to how this product was  
11          going to be used, we'd have to defer to the  
12          clinical or other people within the company.

13       Q.   Oh, no, I understand that. But your idea was,  
14           which was -- I mean, if you disagree let's take a  
15           minute and read it again. But your idea was if we  
16           can get this stuff heated to 60° centigrade for  
17           ten hours or whatever, we may be able to confer a  
18           margin of safety onto this product with respect to  
19           hepatitis?

20       A.   It was my intent at that time in the absence of  
21           data to prove it, yes.

22       Q.   Sure. Tell me, if you can by looking at your  
23           laboratory notebooks, when you started testing the  
24           concept of stabilizing albumin and immune serum  
25           globulin with carbohydrates. When?

- 1     A.    I think if you look to book 2021, page 37.
- 2     Q.    Okay.  This is a -- we will mark it as an exhibit,  
3           but it's got a page number, as you point out, of  
4           37 at the top right-hand corner.  It's in  
5           laboratory book 2021.  It's dated October 25,  
6           1978.  And as we said before with respect to the  
7           other laboratory notebooks of yours, it bears your  
8           signature on the bottom right-hand corner.  Is  
9           that correct?
- 10    A.    Yes.
- 11    Q.    And does this reflect experimental work that you  
12           did on that date?
- 13    A.    It reflects the work that was done as part of this  
14           experiment, but I'm not too sure whether I may  
15           have started the experiment the day before, but I  
16           documented the completion of this experiment on  
17           that day.
- 18    Q.    Okay.  So let's be clear about this.  You may have  
19           started the work on the 24th of October, the  
20           experiment was completed on the 25th of October,  
21           and on the 25th of October you prepared and sent  
22           to the patent counsel what we've marked as Exhibit  
23           3.  Is that correct?
- 24    A.    I believe so.
- 25    Q.    What did the experiment that was completed on

1           October 25th suggest to you?

2       A.    It actually said you could take MISG at 10  
3           dextrose, treat it at 60° -- I think we went for a  
4           little longer time here, fifteen hours -- and  
5           still have it free of opalescence. So it was  
6           essentially clear.

7                    I did make a statement which says,  
8           "Apparently, this heating step can be utilized to  
9           safeguard an IVGG or IMGG," which is an  
10          intravenous or an intramuscular gamma globulin,  
11          "against hepatitis contamination."

12       Q.    So if I understand it, you filed this patent  
13           disclosure on October 25th and you, at that point  
14           in time, hadn't even done any experiments with  
15           albumin or immune serum globulin. Is that  
16           correct?

17       A.    Yes, it is correct.

18       Q.    What was it that led you to suppose that albumin,  
19           a completely different plasma protein, and immune  
20           serum globulin, another completely different  
21           plasma protein, could be stabilized with these  
22           carbohydrates?

23       A.    One of the things that is accepted within the  
24           scientific practice is once one files a patent  
25           application there is several parts to it. One is

1 just a disclosure of the idea, and this represents  
2 the initial disclosure of the idea. This  
3 doesn't --

4 Q. Exhibit 3?

5 A. Yeah. Or this particular page of page 37, or  
6 Exhibit 3, it discloses the concept. There is a  
7 second part to it, which is the reduction to  
8 practice. This is an internal document that says  
9 in my mind I conceived the idea that it is  
10 possible to do the following with this  
11 methodology.

12 Q. No, I think I'm very clear on that. But what I am  
13 trying to find out is what gave you the idea that  
14 stabilizing MISG with these carbohydrates could be  
15 applied to other proteins different than MISG,  
16 like albumin and immune serum globulin?

17 A. I didn't have any specific reason to believe so,  
18 but I made an assumption in the absence of data, I  
19 made the assumption that it may or may not work,  
20 and at this point I said it probably would. The  
21 next step would be to test it out.

22 Q. Sure.

23 A. There was no apriori reason to believe that just  
24 because it had worked on MISG it would have worked  
25 on several others, but I took a guess.

1 Q. You wouldn't know whether they worked or not on  
2 different plasma proteins until you actually did  
3 the experiments. Is that correct?

4 A. Yes.

5 Q. When did you first -- well, before we get too far  
6 ahead of ourselves here, I am going to mark that  
7 page of the laboratory notebook, which has a 37 in  
8 the top right-hand corner, as the next exhibit, I  
9 believe is Plaintiffs' 4.

10

11 MR. JACKSON: And this will be  
12 Plaintiffs' 1000.

13 THE COURT: That's the notebook, or --

14 MR. GOODELL: A page of the notebook.

15 THE COURT: Page of the notebook. Okay.  
16 That's 1000?

17 MR. JACKSON: Offer Plaintiffs' 1000.

18 MR. GOODELL: No objection.

19 THE COURT: One thousand is admitted.

20 MR. JACKSON: And that has the quote I  
21 was looking for. The quote he testified to  
22 earlier was apparently --

23 THE COURT: No, you can't -- it speaks  
24 for itself. Okay?

25 MR. JACKSON: Okay. I just don't know

1 if they can read it.

2 THE COURT: Go ahead. Let's do the  
3 deposition.  
4

5 **DEPOSITION OF DR. FERNANDES RESUMED**

6 Q. Okay. When did you actually begin doing the  
7 experiments with respect to other plasma proteins  
8 and these carbohydrate stabilizers beyond the ones  
9 that you had done with MISG?

10 A. I began to move fairly rapidly from this point,  
11 and I think my first attempt on Koate, which is  
12 really my definition, which is the mix of proteins  
13 that we are talking about, I did that in -- let me  
14 refer to my notebook number.

15 Q. I will tell you what would be helpful to me.  
16 Let's go through -- let's just thumb through these  
17 pages. We don't need to talk about the details of  
18 the experiment, but beginning with page 38, let's  
19 just talk about the plasma proteins you were  
20 working with. So now we are at 38. What proteins  
21 were you working with at that point?

22 A. Here I was looking -- again I was continuing work  
23 on modified immune serum globulins.

24 Q. MISG?

25 A. MISG. On the next page, looking at the functional

- 1 activities of my heat treated gamma globulin,  
2 looking at its potency against polio and measles.
- 3 Q. So you were testing the ability, you were testing  
4 to see whether the product had really been  
5 stabilized, essentially?
- 6 A. Yes. Looking at a characterization of it.
- 7 Q. Again, still MISG?
- 8 A. Still MISG.
- 9 Q. Okay.
- 10 A. Next page, some more data on the viscosities,  
11 densities of MISG.
- 12 Q. Okay. We are on page 41. That's November 7th.  
13 Okay. These are experiments that you are  
14 designing and performing. Is that correct?
- 15 A. Yes, on MISG.
- 16 Q. Forty-two?
- 17 A. On page 41, I begin to look at MISG with  
18 carbohydrates, but also looking at acetyl dL  
19 tryptophan and caprylate as additional stabilizers  
20 to get an effect, to see an effect.
- 21 Q. And those were, in fact, the two stabilizers that  
22 have been well identified for albumin. Is that  
23 right?
- 24 A. Yes.
- 25 Q. Just again so the record is clear, we're at page

1 41 now, which is work done on November 7th. All  
2 right. Continue, please.

3 A. Page 42 is looking at MISG and sucrose, again  
4 doing a trial pasteurization. Page 43 apparently  
5 is an additional experiment looking at  
6 pasteurization with sucrose.

7 Q. Of MISG?

8 A. Of MISG. And page 44 is when I attempt to look at  
9 pasteurization of AHF.

10 Q. All right. Let's mark the pages of the notebook  
11 38 through 43, which covers the period of time,  
12 which covers the days October 31, November 1,  
13 November 7, December 11, December 12 of 1978, as  
14 the next exhibit in order, and that's Plaintiffs'  
15 5 marked for identification.

16

17 THE COURT: Is this 1001?

18 MR. JACKSON: Yes, Your Honor.

19 Additional pages from the lab notebook.

20 THE COURT: Are you going to offer 1001?

21 MR. JACKSON: Yes, we'd offer 1001.

22 THE COURT: Any objection?

23 MR. GOODELL: Are they all ones that are  
24 mentioned here?

25 MR. JACKSON: Yes, sir.

1                   MR. GOODELL: No objection.

2                   THE COURT: One thousand one is  
3                   admitted.  
4

5           **DEPOSITION OF DR. FERNANDES RESUMED**

6           Q.    Let me just ask you this. There does seem to be a  
7                   gap here. I noticed when I was reviewing your  
8                   laboratory notebooks, between November 7th of 1978  
9                   and December 11th of 1978 of a little bit over a  
10                  month.

11          A.    Yeah.

12          Q.    Do you have any idea what that's about?

13          A.    I can't recollect specifically.

14          Q.    Is it possible that you were pulled off of this  
15                  project for a time and helped out in another area  
16                  of the company or something?

17          A.    I really don't know what could have happened.

18          Q.    Well, is it pretty clear, though, that you weren't  
19                  working at the bench during that period of time?

20          A.    It's pretty -- I'm not sure at this time whether  
21                  Amy Loudermilk, who was an assistant to me, or --  
22                  was in the company or not, or whether we were  
23                  working from her notebook or anything else. But  
24                  that's a pure surmise on my part. I don't  
25                  recollect.

1 Q. But the point is if you weren't working out of her  
2 notebook then you weren't doing bench research  
3 between November 7th of '78 and December 11th of  
4 '78.

5 A. On this particular aspect, I could have been  
6 involved in something else at that time.

7 Q. Well, wasn't all of your laboratory work  
8 documented in a notebook?

9 A. Not really. There were two aspects to the work I  
10 was doing. One was this aspect. The other was  
11 also responsible for clinical manufacturing of  
12 gamma globulin which was still going on. In those  
13 particular areas of activity, we had a general  
14 manufacturing batching records in which one would  
15 use those records, which would ultimately go to  
16 the clinical group.

17

18 MR. GOODELL: You said "special  
19 manufacturing".

20 MR. JACKSON: "Special manufacturing  
21 batching".

22 MR. PRICE: Oh, I'm sorry. Let me read  
23 it over.

24

25 DEPOSITION OF DR. FERNANDES RESUMED

1 A. In those particular areas of activity, we had  
2 special manufacturing batching records in which  
3 one would use those records, which would  
4 ultimately go to the clinical group.

5 Q. So you would document your work on the  
6 manufacturing side in batch records?

7 A. Yes.

8 Q. All right. So just to be clear then, unless you  
9 were doing work with Amy and documenting that work  
10 in Amy's lab notebook, you weren't, apparently,  
11 working at the bench on this project between  
12 November 7th of '78 and December 11th of '78. Is  
13 that correct?

14 A. To the best I can recollect.

15 Q. Okay.

16

17 MR. JACKSON: Fernandes Exhibit Number 6  
18 was Plaintiffs' 971. Offer it.

19 THE COURT: What is it?

20 MR. JACKSON: It is a --

21 THE COURT: Notebook page?

22 MR. JACKSON: It's another lab notebook  
23 page.

24 THE COURT: All right.

25 MR. JACKSON: The next exhibit --

1                   THE COURT: Hold it.

2                   MR. GOODELL: No objection.

3                   THE COURT: Nine seventy-one is  
4 admitted.

5                   MR. JACKSON: Thank you, Your Honor.

6                   Page 84, line 5, I'm starting at.

7

8           DEPOSITION OF DR. FERNANDES RESUMED

9           Q.    The next page that I have marked as Plaintiffs'  
10               Exhibit 6 concerns work you confirm performed by  
11               you on December 14th, 1978 is from the lab  
12               notebook 2021, it has a page number 44 at the top  
13               right-hand corner, and it's entitled  
14               Pasteurization of AHF. Is that correct?

15          A.    I want to clarify. You mentioned the wrong month.  
16               It wasn't November. It was December.

17          Q.    Did I say November?

18          A.    Yes.

19          Q.    I didn't mean to. Would you just put December in  
20               there for me? It was December 14th.

21               Well, we have marked the other previous days,  
22               so I'm assuming this experiment must have been  
23               done on December 14th.

24          A.    Again, these are long experiments. Within the  
25               period of a one to two day interval, it was

1 completed. When I did, on the 14th I wrote it  
2 down and said this is what I had done within the  
3 last couple of days.

4 Q. Okay. Because the last page of Exhibit Number 5,  
5 which still documents work done on MISG, is dated  
6 December 12th of 1978, I presumed when I was  
7 looking at these that December 13th and 14th may  
8 have been a weekend or something.

9 A. Well, one of the things we do in, at least one of  
10 the things I do, is you don't write it down  
11 specifically as you are doing every little  
12 manipulation. You do an experiment -- first of  
13 all, different people have different systems.  
14 When I find that this is a meaningful experiment,  
15 I jot it down. In all of this case, that's  
16 essentially how I have done it.

17 Q. Okay. But can we at least agree that this  
18 experiment was completed on December 14th and may  
19 have been started on December 13th?

20 A. Yes.

21 Q. Now, again as with all the others, is this an  
22 experiment that you designed?

23 A. Yes, I did.

24 Q. And before you did this experiment on AHF, did you  
25 consult with Dr. Lundblad?

- 1       A.    I can't recall, looking back.  I was meeting with  
2            him on a weekly basis.  Our offices were next to  
3            each other.
- 4       Q.    Sure.  Did you have the authority, though, on your  
5            own without consulting with him to take a look at  
6            pasteurization of AHF?
- 7       A.    I felt within the organization I was I think  
8            senior enough to initiate some experiments on my  
9            own.
- 10      Q.    Sure.  I'm not questioning that.  But if you told  
11            me that you didn't have that authority that would  
12            tell us both that you probably had to consult with  
13            Dr. Lundblad first.  It may be that you decided on  
14            your own to take a look at the pasteurization of  
15            AHF.  Is that fair?
- 16      A.    Yes.
- 17      Q.    Did anybody at Cutter tell you that don't, you  
18            know, when they saw these disclosures, "don't  
19            bother doing these experiments on AHF", "there is  
20            no way you can pasteurize AHF," or anything of  
21            that nature?  Did anybody suggest that to you,  
22            sir?
- 23      A.    Not really.  I can't recollect them being negative  
24            in that sense of the word.
- 25      Q.    Right.  I mean, it's obvious to me, you tell me if

1 I'm wrong, that very shortly after this experiment  
2 was done other people within the organization  
3 became aware that you were working on  
4 pasteurization of AHF. Is that true?

5 A. It was clear in my mind, as I kept talking to  
6 individuals, that they expressed surprise that  
7 these results were indeed working out, because I  
8 was led to believe -- it was generally accepted  
9 within the scientific community, of which I was an  
10 outsider tot hat, that AHF and a number of other  
11 proteins were not the most stable proteins around.  
12 So they did express surprise.

13 The question then was, which they came back  
14 to me saying, "Well, what does this prove? You've  
15 proved it's not milk. It's not turning milk on  
16 you." But a number of issues needed to be looked  
17 at.

18 Q. Sure. What I'm trying to find out is, did anybody  
19 tell you, "Look, don't waste your time, you can't  
20 stabilize AHF"?

21 A. No.

22 Q. What I was trying to get at is, it's very clear  
23 from the documents we're going to be looking at  
24 that you confirm it probably the day of or the day  
25 after you completed this experiment, others within

1           the research and development department became  
2           aware of your work. Is that correct, sir? With  
3           AHF.

4       A.   With this particular experiment that I had done,  
5           yes, they became aware of it.

6       Q.   Well, is it true that you did no experiments on  
7           AHF whatsoever at Cutter prior to December 14th of  
8           1978?

9       A.   Yes.

10      Q.   This is the very first experiment that you ever  
11           performed on AHF at the bench?

12      A.   To the best of my knowledge, yes.

13      Q.   And did you spend hours or days or months  
14           researching the protein biochemistry literature  
15           before you conducted this experiment?

16      A.   Before I did it, no. Subsequently, yes.

17      Q.   Right.

18      A.   There were issues that needed to be looked at.

19      Q.   Right. But before you did this experiment, that  
20           was maybe started on December 13th and completed  
21           on December 14th, you had not done any scientific  
22           research on AHF or any other coagulation protein.  
23           Is that correct?

24      A.   Well, I want to make it clear in terms of work, I  
25           didn't do any active research but I was a member

1 of the R&D organization at Cutter. There were  
2 weekly meetings that were held, there were people  
3 giving internal talks, and we all had to talk  
4 about our work every so often. So I was aware of  
5 stuff going on, and whatever I have picked up  
6 through osmosis essentially was information that I  
7 retained and is part of my experience base.

8 Q. Sure, and I completely understand that. I assumed  
9 those meetings were taking place. We're going to  
10 talk about them in a minute. But what I was  
11 trying to say is that as you were designing this  
12 experiment on AHF on December 13th or December  
13 14th, you didn't go back into the literature, did  
14 you, and do research on any coagulation proteins  
15 to speak of. Right?

16 A. No, not for this purpose.

17 Q. Or stabilizers, or carbohydrates, or anything of  
18 that nature. Is that right, sir?

19 A. Yes, that is right.

20 Q. Now, at these weekly meetings, let's just talk  
21 about between October and December of 1978, did  
22 you make people within the organization aware that  
23 you had, quote, "surprisingly" discovered that  
24 carbohydrates could stabilize certain plasma  
25 proteins?

- 1       A.    I used to keep my supervisor, John Lundblad,  
2            apprised of day to day activities.  I believe John  
3            had meetings with Milt and the rest of the  
4            research organization.  I was aware of the fact  
5            that they knew what was going on, because John  
6            would come back periodically to me to confirm to  
7            me that people were aware of it, they were excited  
8            about some of this work going on, but that's the  
9            level of communication that was going on during  
10           this time frame.
- 11       Q.    Sure.  Did anyone suggest to you that you ought to  
12            do an experiment to see if this works for AHF?
- 13       A.    Prior to my doing this one?
- 14       Q.    Yes.
- 15       A.    I can't recall, because this was going on so  
16            rapidly.  We're only talking about a sixty day  
17            period from October -- September to December, a  
18            quarter.
- 19       Q.    Well, actually, if we look at these laboratory  
20            notebooks, you confirm it, but we are talking  
21            about approximately six days of bench work, right?  
22            Six days of bench work between October of '78,  
23            when you filed, October -- what day was that first  
24            disclosure?  October 25th of 1978, and December  
25            11th of 1978, we are talking about a total of five

1 or six days at the bench.

2 Well, it's obvious they are all dated. We  
3 are talking about a few days of research at the  
4 bench, aren't we, sir?

5 A. Well, I need to be very clear about this. There  
6 are two aspects to what's going on. One is the  
7 conceptual understanding of how to basically  
8 develop the experiment and design the experiment.  
9 There is a second part of it that is a hands-on  
10 component.

11 It just turned out that the hands-on  
12 component was twelve hours, so it was sitting  
13 there for 12 hours. I don't necessarily assume  
14 that that's all it takes to get the experiment  
15 done. It was the thinking process over the last  
16 six months that was gradually incrementing towards  
17 this ideas.

18 Q. Right. But the experimentation at the bench took  
19 a few days. Right?

20 A. Yes, the mechanics of it took a few days.

21

22 **MR. JACKSON:** Charlie, it's Exhibit 7 to  
23 the Fernandes deposition, which is  
24 Plaintiffs' 291, which is dated 12-10-78,  
25 which we'd also offer into evidence at this

1 time.

2 MR. GOODELL: No objection.

3 THE COURT: Two ninety-one is admitted.

4

5 DEPOSITION OF DR. FERNANDES RESUMED

6 Q. The two disclosures, the one October 25 having to  
7 do with albumin and ISG, and December 14th, 1978,  
8 which I have marked as Exhibit 7, had you looked  
9 at these in preparation for giving your deposition  
10 today?

11 A. I did not specifically read every line of it.

12 Q. No, but did you look? That's why I said "look at  
13 them". Did you look at them?

14 A. Yes. You are talking about the pasteurization of  
15 albumin. I have just read it, yes.

16 Q. In preparation for coming here, had you been  
17 provided with copies?

18 A. Yes, I had been provided with copies.

19 Q. Do you have the one dated December 14th in front  
20 of you?

21 A. No.

22 Q. It's here. I've marked as Exhibit 7, the patent  
23 disclosure dated December 14th, 1978. You are  
24 listed as one of the inventors. It's entitled  
25 Pasteurization of Fibrinogen, Factor VIII (AHF),

1 Factor IX (Konyne), Gamma Globulin, Plasminogen --  
2 what's the next word?

3 (Technical difficulties with ELMO)

4 Q. It's here. I've marked as Exhibit 7, the patent  
5 disclosure dated December 14th, 1978. You are  
6 listed as one of the inventors. It's entitled  
7 Pasteurization of Fibrinogen, Factor VIII (AHF),  
8 Factor IX (Konyne), Gamma Globulin, Plasminogen --  
9 what's the next word?

10 A. "CIG." It stands for Cold Insoluble Globulin.

11 Q. -- and Other Plasma Proteins in the Presence of  
12 Carbohydrates. Right?

13 A. Yes.

14 Q. Now, is it correct that you, as you did in the  
15 previous one in October, you filled out this  
16 disclosure on the very day that you completed your  
17 first AHF experiment. Is that correct?

18 A. Yes.

19 Q. By the way, did you do the assay work yourself on  
20 the first AHF experiment?

21 A. The assay work, referring to this patent, was just  
22 a visual clarity experiment, and yes, that was my  
23 observation.

24 Q. In other words, -- we don't need to cover this  
25 testimony again -- kind of the first step in

1           deciding whether or not this material has been  
2           pasteurized or has been stabilized is to look and  
3           see how clear the material is. Is that right?  
4       A.    Yes.  
5       Q.    You hadn't even assayed AHF at that point. Right?  
6       A.    Not to my knowledge, yes. No, I think we had sent  
7           samples in, yes.  
8       Q.    You sent samples in?  
9       A.    I believe.  
10      Q.    Right. But on December 14th, when you did the  
11           experiment, had you ever in your life performed a  
12           Factor VIII assay?  
13      A.    I have never run the assay myself, and at that  
14           time I had not done it either.  
15      Q.    Did you have the technical competence to run a  
16           Factor VIII assay?  
17      A.    No, I didn't have the technical competence. But  
18           once again, the way we were organized at Cutter,  
19           we had resources available, and it was in the job  
20           to do the experiment and send samples, and I sent  
21           samples to various analytical labs within Cutter  
22           for different purposes.  
23      Q.    Sure. And you would send samples to the assay lab  
24           and they would report back to you what the results  
25           were. Right?

- 1 A. Yes.
- 2 Q. Do you even know what assay was being used back at  
3 this time?
- 4 A. I can't recollect sixteen years, but at that time  
5 I probably -- whatever assay that they felt was  
6 the best assay that was available.
- 7 Q. Sure.
- 8 A. I did look to them to provide me with results. I  
9 took them at face value.
- 10 Q. Right. Absolutely. But the assays for Factor  
11 VIII were reported back to you in terms of what,  
12 activity level?
- 13 A. They were reported back to me in terms of units  
14 per milliliter. It was a volumetric measurement.  
15 I would use that, and I would measure the  
16 absorbance at 280 nanometers, and that would  
17 represent the mass of material, total combined  
18 mass, and I would take a simple ratio of the units  
19 to the mass to give me an indication of activity  
20 present.
- 21 Q. Sure. Now, the people in the analytical  
22 department performing the Factor VIII assays, they  
23 were essentially laboratory technicians, weren't  
24 they? I mean, they didn't have Ph.D's at Cutter  
25 doing Factor VIII assays, did they?

- 1       A.   To my knowledge, this assay wasn't in the quality  
2           control part of the company where routine assays  
3           were done. I was within the research division.  
4           There was an analytical research arm, yes, so they  
5           were scientists running this thing. I was still  
6           dealing within the research part of the  
7           organization. This wasn't standard lab assays.
- 8       Q.   Your testimony is that the persons performing the  
9           actual assays on this material were not  
10          essentially laboratory technicians?
- 11      A.   They were not laboratory technicians.
- 12      Q.   Can you give me the names of any of the persons  
13          who were performing the Factor VIII assays?
- 14      A.   I'm sorry. I can't remember back. But they were  
15          people closely working -- it was part of an  
16          organization run by Doctor -- I think Duane  
17          Schroeder was the head of the research component.  
18          They were people in his lab who were running some  
19          of these assays for me.
- 20      Q.   Do you know what their educational background was  
21          or their experience was? With that --
- 22      A.   I don't know the names of the specific individuals  
23          who ran the assay at that time, but I know they  
24          were part of the research group that did have  
25          experts in the field out there.

- 1 Q. Sure. Now, the disclosure that you prepared on  
2 December 14th, did you confirm that this is in  
3 your handwriting?
- 4 A. Yes, it is.
- 5 Q. What relationship, if any, is there between this  
6 disclosure and the earlier one we discussed dated  
7 October 25th, 1978?
- 8 A. The specific difference is I expanded the initial  
9 disclosure, which dealt with MISG and albumin, and  
10 now saying, well, it may even work on  
11 antihemophilic factor, that mixture that I was  
12 working with. And again, talking about the  
13 conceptual issue, I tried to put as many proteins  
14 that I thought may work or I have got access to.  
15 In the next couple of months, I am going to try  
16 them out.
- 17 Q. Sure. Now, is it true that as of December 14th,  
18 1978, you hadn't even done any experiments to show  
19 that ISG or albumin could be stabilized with these  
20 carbohydrates? That work still hadn't been done?
- 21 A. I knew about MISG, which was done on October 25th.  
22 And albumin, I think there was an experiment which  
23 I tried with albumin.
- 24 Q. I didn't pick up on that. It's Exhibit 5, which  
25 has several pages of laboratory work. I didn't

- 1           pick up an experiment on albumin.
- 2       A.   No, I'm sorry. It was caprylate and acetyl dL
- 3           tryptophan.
- 4       Q.   On MISG?
- 5       A.   You are right.
- 6       Q.   So you had worked with MISG, but only MISG.
- 7           Correct? Not albumin or ISG. Right?
- 8       A.   Let me go back specifically and look at some of
- 9           these pages. There is an experiment on page 38 in
- 10          which I had 5% albumin and 10% dextrose and showed
- 11          that it was clear on heat treatment.
- 12       Q.   Well, was that adding the 5% albumin to the MISG
- 13          preparation?
- 14       A.   No, it was 5% albumin and 10% dextrose.
- 15       Q.   And which day was that?
- 16       A.   That's on page 38. It says October 31, I think.
- 17          Yeah.
- 18       Q.   Which is part of Exhibit 5?
- 19       A.   Yes.
- 20       Q.   Okay. So you had done an experiment on albumin on
- 21          October 31st. Is that correct?
- 22       A.   Yes.
- 23       Q.   Okay. Now, at the time you filed the patent
- 24          disclosure with Cutter's Patent Office, patent
- 25          counsel rather, on December 14th, what, if any,

1 testing of stabilization had you done on  
2 fibrinogen and all of these other plasma proteins  
3 identified in the disclosure?

4 A. I don't think I had done any of them as of then.

5 Q. Tell me what it was that caused you to think  
6 conceptually that these carbohydrates that you are  
7 discussing could potentially stabilize these other  
8 completely different plasma proteins? Well, let's  
9 cover that. I mean to make it clear to the Jury.

10 These proteins that you are identifying in  
11 the disclosure dated December 14th are completely  
12 different proteins from albumin and MISG and ISG,  
13 aren't they?

14 A. Right.

15 Q. In fact, these are coagulation proteins, and the  
16 other proteins you were working with were not  
17 coagulation proteins?

18 A. Right.

19 Q. So conceptually you were thinking, "If I can use  
20 these carbohydrates to stabilize noncoagulation  
21 proteins, maybe I can use them to stabilize  
22 coagulation proteins." Is that correct, sir?

23 A. No, that wasn't the way I saw it. I think at this  
24 time my mental state was, I was excited about  
25 this. I was very, very naive in terms of what can

1 or cannot be done. I came out, I wrote a patent  
2 disclosure dealing with the full range of  
3 proteins. As it turned out subsequently to this,  
4 I found out that fibrinogen, for example, cannot  
5 be pasteurized, and I had to get back to the  
6 patent people and take that one off.

7 Q. Sure.

8 A. So, yes. But at that point I said, "Let me lay  
9 out the ground work."

10 Q. But if I'm -- stick with me here for a second. If  
11 I'm thinking about this the way you were, these  
12 carbohydrates were, that you had worked with  
13 previously, were not coagulation proteins. Is  
14 that right? These proteins that you were working  
15 with, MISG and albumin, were not coagulation  
16 proteins, were they?

17 A. No, they were not.

18 Q. Well, what led you to believe that the work that  
19 you had done on these noncoagulation proteins  
20 could potentially be used to pasteurize  
21 coagulation proteins?

22 A. I don't think I have anything more than what I  
23 just said.

24 Q. Why did you believe that sucrose could potentially  
25 stabilize AHF?

- 1     A.   I didn't believe at that time that sucrose could  
2           stabilize AHF. This was an empirical analysis  
3           that came into the picture. I wasn't really  
4           separating coagulation proteins from  
5           noncoagulation proteins in the way I was looking  
6           at this. I was looking at all the proteins that  
7           were available and saying, "I've got some data  
8           that says I can stabilize MISG. I've got some  
9           information that says that sucrose seems to work  
10          on AHF. I'm going to look at the other proteins  
11          that I've got access to in the company." That was  
12          my reasoning.
- 13    Q.   Right. But what was it that caused you to use a  
14          particular carbohydrate to do an experiment on  
15          AHF, which was a totally different protein than  
16          the MISG or albumin you had been working with?
- 17    A.   I've --
- 18    Q.   Well, let me put it this way. You couldn't  
19          possibly know whether any of these carbohydrates  
20          would work with any of these proteins without  
21          doing the experiment. Right?
- 22    A.   Yes.
- 23    Q.   I mean, you couldn't. Just because it worked with  
24          MISG didn't mean it would work with AHF. You have  
25          to do an experiment with AHF. Right?

1 A. Yes. I need to -- in the most respectful way, I  
2 mean, it works something like this.

3

4 MR. JACKSON: Sometimes.

5 MR. PRICE: I'm sorry.

6

7 DEPOSITION OF DR. FERNANDES RESUMED

8 A. It works sometimes like this. I mean, I didn't  
9 have a preconceived reason, went into the lab,  
10 there are these things sitting out there, I picked  
11 them and did it. I mean, that's the level, as you  
12 see, a lot of these experiments worked out.

13 Q. No, I understand that. But what I am trying to  
14 say is you surmised here in your disclosure that  
15 these other plasma proteins, some of which are  
16 coagulation proteins, could be stabilized, but you  
17 wouldn't know which carbohydrate would work, at  
18 what concentration, without actually doing the  
19 experiment. Correct? Well, isn't that right,  
20 sir?

21 A. What I actually said, at that point I made an  
22 assumption that these would work on those  
23 proteins.

24 Q. But you wouldn't know they worked until you  
25 actually did the experiment. Right?

1       A.    Yes.  I think it would not confirm the accuracy of  
2            my assumptions until I have done it, yes.

3

4            THE COURT:  Mr. Jackson, let me ask a  
5            question.  How close are we?  Because we're  
6            going to have to do some things.

7            MR. JACKSON:  Probably still forty --

8            MR. PRICE:  It's as good a place to  
9            break as any, Your Honor.

10           MR. JACKSON:  Forty-five minutes,  
11           probably.

12           THE COURT:  Okay.  Then pick a spot.  Is  
13           this a good place to break or not a good  
14           place to break?

15           MR. PRICE:  I think this is a good  
16           place.

17           MR. JACKSON:  Whatever the witness says,  
18           Your Honor.

19           THE COURT:  All right.  Then we'll do  
20           that because we've got to do a couple of  
21           things.

22           So, all right.  Then we'll come back and  
23           hear the rest of Dr. Fernandes' testimony  
24           tomorrow morning.  So with that, I'm going to  
25           go ahead and excuse you until 8:50 tomorrow

1 morning.

2

3

WHEREUPON THE JURY WAS ADMONISHED AND  
EXCUSED FROM THE COURTROOM

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1     THURSDAY, FEBRUARY 13, 1997:

2

3     DEPOSITION OF DR. FERNANDES RESUMED

4     Q.    Sure.  That's all I'm trying to say.  Because at  
5           this point when you filed the disclosure you  
6           hadn't done any experiments on any of these.

7     A.    Yes.

8     Q.    Except for AHF.  Right?

9     A.    Well, as of December 14th, yes.

10    Q.    Why did you choose AHF as the first coagulation  
11           protein to do the experiment on?

12    A.    For no specific reason, other than the fact that  
13           AHF was more talked about in the company than  
14           Factor IX or fibrinogen or any one of the reagents  
15           -- I'm sorry -- any one of the other proteins that  
16           we were working on.

17    Q.    Well, weren't you aware when you did this  
18           experiment on December 14th that virtually every  
19           user of AHF, that is of your company's products,  
20           was being infected with hepatitis?

21    A.    I had no awareness of that at that time.

22    Q.    Well, were you aware that AHF was capable of  
23           transmitting hepatitis in December of 1978?

24    A.    I was aware of the fact that there was the  
25           potential, because this material -- I was aware of

1           the fact that Fraction 1 and some of the  
2           components present in the initial steps of plasma  
3           processing could contain the hepatitis virus. I  
4           was aware of that, or have some infective  
5           component to it.

6       Q.   And could potentially infect a recipient of the  
7           product?

8       A.   Yes, I believe it was an issue, apparently it was  
9           an issue of risk and benefit. It wasn't in my  
10          area of expertise. I picked it up because it was  
11          there.

12      Q.   I understand that. And I'm trying to find out --  
13           and all I am trying to find out is, when you were  
14           deciding to prioritize which of these plasma  
15           proteins to experiment on first, did you take into  
16           consideration the fact that virtually every  
17           recipient of AHF was being infected with  
18           hepatitis?

19      A.   No, I did not.

20      Q.   Were you instructed by anyone within the company  
21           to give first priority to AHF for any reason  
22           having to do with its potential safety from a  
23           viral standpoint?

24      A.   No.

25

1                    MR. JACKSON: One-0-five, 2.

2

3                    **DEPOSITION OF DR. FERNANDES RESUMED**

4                    Q.    Dr. Fernandes, the second page of the December  
5                                14th disclosure references a paper by Seegers. Do  
6                                you see that?

7                    A.    Yes, I do.

8                    Q.    When do you believe that you first reviewed the  
9                                Seegers paper?

10                  A.    I do not recollect a specific date, but I think it  
11                                was sometime during the October-December time  
12                                frame.

13                  Q.    Do you know how you got to the Seegers article?  
14                                Did somebody within your department mention it to  
15                                you, or did you do independent research, or what?

16                  A.    I don't remember specifically. I think I had a  
17                                handle, you know, after going through most of '78  
18                                on carbohydrates, getting closer and closer, and  
19                                I'm not too sure what was my anchor that led me to  
20                                that particular thing, because this article is not  
21                                -- it's an old one, and it was referred by  
22                                something. I can't track back that level of  
23                                detail.

24                  Q.    Okay. Is it correct that the Seegers article  
25                                deals with thrombin?

- 1 A. Yes.
- 2 Q. And thrombin is a plasma protein, is it?
- 3 A. Yes, it is.
- 4 Q. Is it a coagulation protein?
- 5 A. I'm not really an expert in this area. Mine would  
6 be a layman-ish view. Yes, it's involved  
7 somewhere in the coagulation cascade.
- 8 Q. You don't consider yourself a so-called  
9 coagulation scientist, do you?
- 10 A. Oh, no, by no means, no.
- 11 Q. Did you prepare this schematic?
- 12 A. Yes, I did.
- 13 Q. Yourself?
- 14 A. Yes, I did.
- 15 Q. And I take it that this came out of your  
16 knowledge, generally speaking, of the  
17 manufacturing process for the various proteins?
- 18 A. Well, I hypothesized a certain process that I  
19 think could have worked.
- 20 Q. Right. When you were conceiving this invention,  
21 did your conception include removing the  
22 stabilizers at some point during the process?
- 23 A. Yes, it was very clear in my mind that that would  
24 be a necessary condition to make it work.
- 25 Q. Right. And what mechanisms were you thinking

- 1           about for removing the carbohydrates?
- 2       A.    The most obvious method would be to consider some  
3           kind of dialysis or diafiltration.
- 4       Q.    Okay.  There is a sentence here at the bottom  
5           which I'd like to read in for the record and then  
6           ask you some questions about.  It says, quote,  
7           "Since this is a bulk pasteurization step, all  
8           processing on the downstream side would have to be  
9           done in a hepatitis free area," end of quote.  
10          First of all, "bulk pasteurization" meaning what?
- 11      A.    Bulk pasteurization is pasteurization in a large  
12           bulk of liquid as opposed to what's done in  
13           albumin, where you could pasteurize it in its  
14           final container vial.
- 15      Q.    Why in a hepatitis-free area?
- 16      A.    Well, the concerns I had then was that we were  
17           going through the process specifically to reduce  
18           the level of hepatitis.  The last thing we would  
19           want to have happen is that because it's being  
20           done in the bulk you've got to remove it and  
21           you've got to handle it, put it into final vials.  
22           You don't want contamination coming in after the  
23           process.  In my mind, that's just good practice.
- 24      Q.    Sure.  The next sentence reads, "It should be  
25           noted that all carbohydrates could theoretically

1           be used to protect the protein during  
2           pasteurization," end of quote. Were you referring  
3           to the proteins that you listed on the front page  
4           of the disclosure?

5       A.   Implicitly, probably those proteins.

6       Q.   And what carbohydrates were you thinking about?  
7           You say "all carbohydrates." Which ones did you  
8           know about in December of 1978 which you believed  
9           could potentially stabilize these plasma proteins?

10      A.   At this point, this was a statement made without  
11           any particular goal in mind. It was a concluding  
12           statement saying, I think there is something to  
13           these carbohydrates that may be helpful here.

14      Q.   Well, what carbohydrates had you been working  
15           with?

16      A.   Historically, I had looked at fructose, sucrose,  
17           mannitol, maltose. Those are probably the range.  
18           So I used some of those.

19      Q.   Did you go back into the literature and look at  
20           some of the work that had been done with those  
21           stabilizers, those carbohydrates?

22      A.   Not really. Again, what was motivating me, after  
23           having got a handle here, my goal was to push this  
24           thing through, you know, and try to do some  
25           experiments or confirm. It wasn't necessary for

1 my aspect of the program.

2 Q. To do the research. Right?

3 A. Yeah.

4 Q. Okay. Let's get back, if we can then, to the

5 notebooks. It looks like --

6 A. Page 45.

7 Q. -- we're on page 45 now. It looks like you are

8 continuing your experiments on AHF on the December

9 18th -- go ahead.

10 A. Well, one of the things that came to mind was if

11 you look on December 14th I picked one particular

12 batch M5138, which was a random batch of material

13 I picked up from the inventory that existed. I

14 picked it, I did it, it gave me a good idea to put

15 it in the disclosures. I went back to try to

16 confirm it and pick some other material, in this

17 case it was C1051, and it failed on me.

18 Q. Which are you --

19 A. On the third line, which says it was pasteurized

20 from 9:30 p.m. to 7:20 a.m. the following day, and

21 the material was observed to come out of solution.

22 Q. Right. And that was with 50 grams of sucrose?

23 A. Sucrose, and about 60 mls --

24

25 MR. PRICE: I guess that's milliliters.

1 DEPOSITION OF DR. FERNANDES RESUMED

2 A. -- of AHF solution.

3 Q. Is it 60? Or total volume was 86 mls, but 60 mls  
4 of it was AHF?

5 A. Yeah, I think there was some volume expansion. I  
6 ended up with a volume of 86 mls.

7 Q. By the way, that particular experiment, we're  
8 looking at a notebook dated December 18th, 1978  
9 with a page number in the right-hand corner of 45  
10 that says, quote, "AHF sterile scrap." What does  
11 that mean?

12

13 MR. PRICE: Do you want to put that  
14 exhibit up?

15 MR. JACKSON: I don't think he's  
16 identified it yet. Give me a second. I'll  
17 see.

18 MR. PRICE: It says it's page 45.

19 MR. JACKSON: That's Plaintiffs' 972,  
20 which we'll go ahead and offer.

21 THE COURT: This is a notebook page?

22 MR. JACKSON: Yes, sir.

23 MR. GOODELL: No objection.

24 THE COURT: Exhibit 972 is admitted.

25 MR. JACKSON: At line 20, that same

1 question again.

2

3 DEPOSITION OF DR. FERNANDES RESUMED

4 Q. By the way, that particular experiment, we're  
5 looking at a notebook dated December 18th, 1978  
6 with a page number in the right-hand corner of 45  
7 that says, quote, "AHF sterile scrap." What does  
8 that mean?

9 A. I think this was material that what happens in the  
10 manufacturing process, one gets your product, one  
11 takes a certain fraction of the material to do a  
12 lot of analytical testing for a specific need.

13 In this particular case, and it's hard for me  
14 to remember back, but it would mean that samples  
15 which were taken originally to measure the  
16 existent sterility, there may be some extra vials  
17 that were housed in inventory which I picked up.

18 Q. Do you know how old the material was?

19 A. I can't recollect at this point what the age of  
20 the material was, but this was inventory that  
21 people sent out for research purposes.

22 Q. Sure. So if you can, just beginning December 18th  
23 of 1978, just kind of flip through some of these  
24 pages and give me an idea generally what kind of  
25 experiments you were doing.

- 1 A. I was beginning to take some trial shots at  
2 Konyne, and it's hard for me to recollect from  
3 these notebooks, but I don't think I saw  
4 significant results and I passed on. Forty-six  
5 deals with some information on MISG and maltose,  
6 again looking at some analytical characterization.
- 7 Q. Okay. You got back to AHF, it looks like, on the  
8 next page, 47.
- 9 A. I was going back and forth looking at different  
10 batches at this time. And 5368, there is no  
11 precise meaning to those numbers.
- 12 Q. What kind of results did you get in those  
13 experiments?
- 14 A. Well, in those experiments, it looked like adding  
15 different levels of sucrose I was getting clear  
16 material after close to 22 hours, so this was an  
17 expanded pasteurization condition. So these are  
18 what I would consider good results initially.
- 19 Q. Right. Just again so the Jury will understand,  
20 you were doing these heating experiments at a  
21 constant temperature of 60° centigrade for 22 and  
22 a half hours, but varying the concentration of  
23 sucrose. Is that right?
- 24 A. In this particular experiment, yes, I think that's  
25 what we were doing.

- 1 Q. The next page it looks like again more experiments  
2 on January 2nd. It looks like, correct me if I'm  
3 wrong, I'm trying to speed through this, that you  
4 were doing work on ISG and AHF. Is that right?
- 5 A. Yes. I was moving into the area now of removing  
6 sucrose, beginning experiments dealing with  
7 dialysis or diafiltration. I think in this case  
8 it was dialysis.
- 9 Q. And that was work, if I didn't say it before, on  
10 January 2nd, lab notebook page 48. What was the  
11 purpose of adding the glycine and the sodium  
12 chloride?
- 13 A. In those days, final container antihemophilic  
14 factor was present with these excipients in it.  
15 So what I was trying to do was to diafilter the  
16 sucrose containing material into the final  
17 excipients that the standard AHF had.
- 18 Q. So in your design of these experiments, you were  
19 trying to get as close as you could to actual  
20 manufacturing process?
- 21 A. Yes, in a sense I was removing sucrose and  
22 increasing the final container buffers.
- 23 Q. Right. Let's go to the next page. Now, here it  
24 looks like somebody has given you results back  
25 maybe?

- 1 A. Yes.
- 2 Q. What results are those giving?
- 3 A. I had given samples of the feed material prior to  
4 pasteurization and samples post-pasteurization. I  
5 got back results which said the feed was 27 units  
6 per ml, and the pasteurized material apparently is  
7 2.8 units per ml now.
- 8 As I began to remove the sucrose and add back  
9 the final container excipients, you get a change  
10 in volume. You are not able to control the volume  
11 during dialysis. So I did measure the mass  
12 content of the material, and that's defined by an  
13 A280 reading, and I essentially took the ratios of  
14 post to pre and said, well, it looks like we are  
15 getting close to 45% recovery.
- 16 Q. That's the recovery of AHF across the  
17 pasteurization step?
- 18 A. Yes, just across the pasteurization and removal  
19 step.
- 20 Q. So if I'm correct, and you tell me if I'm wrong,  
21 the loss of activity was the result of a  
22 combination of pasteurization and removal of the  
23 sucrose. Correct?
- 24 A. That would be the implication.
- 25 Q. Right. Now, also on January 4th it looks like you

1 began working with pasteurization of defatted  
2 albumin. Is that right?

3 A. Yes, it is.

4 Q. Just follow with me here. On January 5th, we're  
5 now at page 50 of your laboratory notebook, you  
6 went back to AHF. Is that right?

7 A. Yes, I did.

8 Q. And what is -- at the top left-hand corner, it  
9 says CAE analysis. What is that?

10 A. That CAE stands for cellulose acetate  
11 electrophoresis, and it's a method used to  
12 separate out the components of any mixture. It's  
13 a charge separation.

14 Q. And explain to me what this little chart means  
15 that's at the top left-hand corner.

16 A. The concern I had, as I mentioned in the previous  
17 answer, that it seemed that the pasteurization  
18 seemed to work on one batch and did not work on a  
19 second batch. That was troubling me a little bit,  
20 and so one of the things I did was to say what's  
21 the best way to figure this out but to run an  
22 electrophoretic pattern on each one of the lots  
23 and see if I could see any gross differences.

24 The first items say albumin. Albumin is used  
25 as a control in those assays. It migrates to a

1           certain position, and you say okay, assay is  
2           working.

3           So I looked at these batches of material, and  
4           then looked at the ratios. It looked like there  
5           were different levels of fibrinogen as well as  
6           beta globulins.

7           You've got to recognize that AHF, unlike my  
8           experience with MISG, it's a mix of proteins. So  
9           I was looking at gross differences in a purely  
10          empirical way, saying it looks like C1051, which I  
11          had shown in the past had failed, say well, it  
12          looks like it has a high level of fibrinogen than  
13          the earlier batch. It was an observation that I  
14          noted mentally.

15        Q. Now, again, I want to try, if I can, to put this  
16          in lay language so the Jury understands. When you  
17          got different runs on these two lots, your  
18          question was, is there something in these two  
19          different batches that would account for the  
20          difference in results that I was getting? Is that  
21          right?

22        A. Yes, it was a combination. There was something  
23          different in the experiments I had done, a major  
24          fraction of it was the components of the mixture.

25        Q. So the first thing you did was to see, let me test

1           these two different lots and see if the protein  
2           content is different, one between the other.  
3           Right?

4       A.    Yes.

5       Q.    And you identified, when you did the experiment on  
6           January 5th of 1979, that there was a difference  
7           in fibrinogen content between the two lots. Is  
8           that right?

9       A.    Yes.

10      Q.    And so then you decided to do an experiment on  
11           January 5th to test out the effect of fibrinogen  
12           content on the stabilization of AHF. Correct?

13      A.    Yes.

14      Q.    Is the answer yes?

15      A.    I'm sorry. This was the effect of fibrinogen  
16           pasteurization by itself at this point, yeah.

17      Q.    Okay. In other words, you decided to look at  
18           fibrinogen, different quantities of fibrinogen, to  
19           see what the effect was over the pasteurization  
20           step?

21      A.    Yes, that's on a subsequent experiment. On page  
22           50 was taking a vial of -- now, this is Parenogen,  
23           another product.

24      Q.    I'm not there yet. I will get to that, because  
25           I'm sensitive to what your lawyer wants to do

- 1           here. We are going to mark these as exhibits, by  
2           the way, so it's clear.
- 3       Q.   There are really two separate experiments going on  
4           here on page 50. Right?
- 5       A.   Yes, there are.
- 6       Q.   The first experiment on page 50 is the one we've  
7           been discussing, and that's where you looked at  
8           the protein content of two different lots of AHF.
- 9       A.   Yes.
- 10      Q.   The lot that seemed to work on stabilization and  
11           the lot that seemed not to work on stabilization.  
12           Right?
- 13      A.   In general, yes.
- 14      Q.   And the lot that worked on stabilization you found  
15           that the fibrinogen content was in the low 30%  
16           range, and the lot that didn't work you found the  
17           fibrinogen content was in the 60% range or so. Is  
18           that correct, sir?
- 19      A.   Yes.
- 20      Q.   All right. So that led you to believe that maybe  
21           the fibrinogen content had something to do with  
22           whether the material could be stabilized?
- 23      A.   At this point, it was either the fibrinogen  
24           content or the ratio of mixes of whatever was in  
25           there. The only thing I could measure was the

- 1           fibrinogen.
- 2       Q.   And it looks like you measured something else.  Is
- 3           that globulin?
- 4       A.   It's beta globulin that exists.
- 5       Q.   So you were able to measure fibrinogen content and
- 6           beta globulin content?
- 7       A.   Yes.
- 8       Q.   All right.  Why did you assume that it was the
- 9           fibrinogen content and not the beta globulin
- 10          content that affected the ability of AHF to be
- 11          stabilized?
- 12      A.   Again, this is based on purely empirical method.
- 13           I had a vial of fibrinogen available.  There was
- 14          no beta globulin available in the company, so I
- 15          accepted what was available and began testing it.
- 16      Q.   All right.  So it was just a question of you were
- 17          able to get fibrinogen to work with --
- 18      A.   If beta globulin had been available, I would have
- 19          tested that, also.
- 20      Q.   Okay.  Now, the second experiment that's
- 21          identified on page 50 of the notebook is an
- 22          experiment you did with Parenogen.  Correct?
- 23      A.   Yes.
- 24      Q.   I think Parenogen refers to a fibrinogen material.
- 25          Is that right?

- 1 A. I think that was a trade name for Cutter's  
2 fibrinogen at that time.
- 3 Q. Was that an actual product that was being sold by  
4 Cutter?
- 5 A. I do not recollect whether they were selling it at  
6 that time. I knew they had manufactured batches  
7 of this material at some time.
- 8 Q. Right. Well, it had an expiration date of October  
9 17th of 1974. Right?
- 10 A. Yes, it did.
- 11 Q. Did that mean the product was outdated?
- 12 A. From a marketing sense, yes, it was outdated.
- 13 Q. All right. So just in lay terms, as best you can,  
14 explain the work that you did with the fibrinogen  
15 product.
- 16 A. What I did was to take a vial of fibrinogen,  
17 reconstitute it, add sucrose to it according to  
18 the recipe I had been working on, and try to  
19 pasteurize it, and I think noticed that it very  
20 rapidly jelled. In fact, it jelled within a half  
21 hour at 60° centigrade.
- 22 Q. What did that add to your knowledge of the  
23 potential for fibrinogen to be affecting the  
24 ability to pasteurize AHF?
- 25 A. The first thing that came to mind, again saying if

1           fibrinogen is coming out of solution, it's  
2           probably dragging other stuff also with it. And  
3           in fact, what I'm really seeing when a vial gels  
4           is really the fibrinogen jelling with the AHF in  
5           the mix, or whatever.

6       Q.   All right. I realize this is just one experiment,  
7           but it kind of got you thinking that maybe the  
8           jellation of the fibrinogen during pasteurization  
9           may have affected the activity of Factor VIII?

10      A.   Well, I'm not sure at that time I was that  
11           eloquent in my analysis. I just assumed that we  
12           had a problem here that fibrinogen components in  
13           the AHF was something that we had to get rid of.

14      Q.   Let's mark these laboratory notebook pages that  
15           we've been discussing as the next exhibit. They  
16           go from 45 through 50, and cover the days December  
17           8th, December 18th of '78, January 2nd, '79,  
18           January 14th, '79, and January 5th, '79. I  
19           believe that will be Exhibit 8.

20

21                   **MR. PRICE:** I think you misread. It's  
22           January 4th, not January 14th.

23                   **MR. JACKSON:** Okay. "January 4th and  
24           January 5th of '79. And I believe that would  
25           be Exhibit 8."

1                   MR. PRICE: And those are all exhibits?

2                   MR. JACKSON: Those are the ones we've  
3                   been discussing.

4

5           **DEPOSITION OF DR. FERNANDES RESUMED**

6           Q. Does it appear in your laboratory notebook 2021  
7           that you continued, though, to evaluate the effect  
8           of fibrinogen content on January 8th, 1979?

9           A. Yes, it does.

10          Q. And what did that add to your knowledge as to the  
11          potential effect of fibrinogen content on  
12          pasteurization of AHF?

13          A. What was going through my mind was, again, trying  
14          to quantify these issues as best that I could.  
15          You have got to recognize, again as background  
16          material, I was coming from an MISG program where  
17          working with close to 100% pure material I could  
18          do all sorts of things with it.

19                 Here, looking at -- I went back to purely  
20          empirical analysis. Here I've got a vial which is  
21          supposedly 100% fibrinogen, and it gels in 30  
22          minutes. I've got some AHF on this side which I  
23          know at some level turns out to be 31, 33%, I'm  
24          going to start missing. I'm going to do an add  
25          back experiment.

1           So I took a vial of fibrinogen and added  
2           different levels of AHF, or maybe the other way  
3           around, but tried to cover the range, and the  
4           range I could cover would be the minimum level of  
5           fibrinogen and AHF, which was somewhere 30, 33%,  
6           and of course you got close to 94% of fibrinogen  
7           on that end, and began to see whether I could see  
8           a correlation between that and material coming out  
9           of solution. And indeed, again, looking at it,  
10          there is an empirical relationship.

11          At this point I wasn't interested in causes  
12          or effects or mechanisms, an observation that as  
13          you increase the ratio of fibrinogen in your mix  
14          of AHF it's going to come out of solution faster.

15        Q.   Again to put it in lay terms, the more fibrinogen  
16              that was in the mixture you were working with, the  
17              less Factor VIII activity you had at the end of  
18              the pasteurization step?

19        A.   Well, at this point, I mean, the activity  
20              measurements would have come subsequently to this.  
21              Again, I was looking purely at opalescence. This  
22              was an easy thing I could do on-site, and my  
23              initial observations were focused primarily on  
24              opalescence rather than on activity measurements  
25              at this point.

- 1 Q. So, in other words, when you had less fibrinogen  
2 content at the end of the pasteurization process,  
3 you had a clearer looking material than you had  
4 when you had more fibrinogen content, and at the  
5 end of that process the material was cloudy. Is  
6 that what you mean?
- 7 A. Yes, that's the interpretation.
- 8 Q. And you were surmising that the appearance of the  
9 material could relate in some way to the  
10 destabilization of Factor VIII?
- 11 Q. In other words, the cloudy material perhaps  
12 meaning that the Factor VIII had not been  
13 stabilized and the clear material meaning that  
14 perhaps the Factor VIII had been stabilized?
- 15 A. In layman's terms, it wasn't a good sign to see  
16 this happen with the increasing levels of  
17 fibrinogen.
- 18 Q. The cloudiness?
- 19 A. The cloudiness.
- 20 Q. Okay. Continuing then with your lab notebooks,  
21 and we are not going to go through all of this in  
22 this kind of detail, but it looks like on page 52  
23 you looked at pasteurization of Konyne. Is that  
24 right?
- 25 A. Yes, I did.

1 Q. And describe just in brief detail the experiment,  
2 or the experiments, that were going on on page 53  
3 of your notebook.

4 A. On 53 or 52?

5 Q. 52 is Konyne, and I'm skipping over that for the  
6 time being. It looked to me, correct me if I'm  
7 wrong, that your initial work on Konyne in terms  
8 of pasteurization was not altogether successful.  
9 Is that correct?

10 A. Yes. Failed.

11 Q. Failed. So let's go to page 53, when we're back  
12 working with AHF. Just kind of describe for me  
13 what you were doing at this point.  
14

15 MR. JACKSON: This will be Plaintiffs'  
16 973. It's just a continuation of the lab  
17 notebook.

18 MR. PRICE: What page is that?

19 MR. JACKSON: That's starting at page 51  
20 of the lab notebook. The exhibit number is  
21 973, which we would offer into evidence.

22 MR. GOODELL: No objection.

23 THE COURT: Exhibit 973 is admitted.  
24

25 DEPOSITION OF DR. FERNANDES RESUMED

1     A.    I was trying to continue to begin to understand  
2           this issue of reproducibility, and in fact, this  
3           one aspect of it that plagued me for most of that  
4           quarter, an ability to come back and in some  
5           confident way and say look here, this is the  
6           recipe to pasteurize material, it worked  
7           sometimes, it won't work sometimes.

8                 What you see is a series of experiments, and  
9                 again, as a fact of life, I have not put all the  
10                information in some of these notebooks, but I can  
11                tell you in concept the more I started working  
12                with this protein the harder things started to  
13                happen.

14               One of the things I realized also is that I  
15                was initially looking at just 60° for 10 hours and  
16                looking at the material after pasteurization, and  
17                if it looked clear I would say good, and I'd move  
18                in a particular path and then realize that even  
19                though it was clear during the heat treatment  
20                step, as I removed the sucrose, material could  
21                come out of solution.

22               So here we were looking at multiple forms of  
23                instability. There was one form of instability  
24                against heat, but there was another form of  
25                instability which was more geared towards

1 solubility.

2 So looking at multiple mechanisms of  
3 denaturation occurring, the difficulty and the  
4 frustration I felt in a lot of this stuff was  
5 again going back to other proteins. Here I was  
6 dealing with an activity measurement that I was  
7 getting, no other way to say, is it unfolding? Or  
8 sulfhydryl groups that were being exchanged to  
9 give me a handle as to what is the right path.

10 But during this period -- and I think most  
11 of the work during the month of January was really  
12 work trying to figure my way to not only this  
13 concept of nonreproducibility at the high  
14 temperature and reproducibility of removal of  
15 sucrose, as we call that.

16 Q. And would it be fair to say that the work that you  
17 were doing in the month of January represented  
18 systematic experimentation where you were trying  
19 to optimize essentially the pasteurization of AHF?

20 A. I was trying my best under the circumstances to do  
21 a systematic type of experiment, but the  
22 frustration that I was facing is really in terms  
23 -- as a bench scientist now, as a person in the  
24 lab trying to figure this thing out, the lack of  
25 tools, yes, there was an activity number that I

1           was being given, and I had the visual observation  
2           of cloudiness, and trying to work between those  
3           parameters, yes.

4       Q.   Right.  So let's just kind of go through this for  
5           a minute.  I was asking you -- I think when I  
6           started this conversation I was asking you about  
7           what happened on January 10th of 1979.

8       A.   Right.

9       Q.   Is this the first time you began sending samples  
10          to the coagulation -- to the assay people to have  
11          work done on a material that had been pasteurized?

12      A.   I think there may have been one or two occasions  
13          in the past where I reported around 45%.

14      Q.   That's right.

15      A.   The difficulty we were having in a purely  
16          practical way is my initial samples were at 60%  
17          sucrose, or saturated levels of sucrose.  That's a  
18          viscous material, and ability to siphon out a  
19          microliter, or two microliters is unfortunately a  
20          mundane but real problem, and there were issues  
21          connected by the lab people coming back to me and  
22          saying, "Get the sucrose out before you give it to  
23          me.  So there were those sorts of day-to-day  
24          issues that needed to be sorted out.

25      Q.   Sure.  Now, did these samples on January 10th get

1 sent for analysis?

2 A. I think this is part of an experiment that  
3 continues on the next page in which yes, I think  
4 we started getting some responses back from the  
5 analytical lab.

6

7 MR. GOODELL: I've lost where you are.

8 Would you mind --

9 MR. JACKSON: Yeah.

10 MR. GOODELL: What page?

11 MR. PRICE: It's at page 125, line 25.

12 MR. GOODELL: Thank you.

13

14 **DEPOSITION OF DR. FERNANDES RESUMED**

15 Q. And what kind of responses were you getting? Just  
16 kind of summarize.

17 A. Well, you can see the feed I had arbitrarily  
18 assigned as 100%, and I was seeing close to 48, if  
19 you look in the left-hand side, about 48% recovery  
20 in terms of the heat treated portion of it.

21 I was also showing simultaneously as a  
22 control sample, if I just left the sample at 5% in  
23 a refrigerated condition under these same  
24 conditions, as a control I was seeing 68%  
25 recovery, which meant that just the mere act of

1           letting this material sit in the refrigerator was  
2           somehow giving me a lower number. That sort of  
3           confused the issue a little bit in my mind talking  
4           about recoveries.

5       Q.   Right.

6       A.   I'm not implying -- we were using whatever assays  
7           were available, and I was not about to do anything  
8           else except report the results, but there was a  
9           sense of frustration coming through.

10      Q.   Sure.

11      A.   I could also say that when I dialyzed the sample I  
12           got an observed result of close to 87% recovery.  
13           Now, again that ties into the fact that somehow  
14           the activity had gone up after this pasteurization  
15           step. Is it due to removal of sucrose? Is it due  
16           to just the reliability of assay numbers? As I  
17           continued to plow on with this, hoping that things  
18           would work out as we would proceed, but a sense of  
19           frustration was creeping into my analysis and  
20           thinking.

21      Q.   Sure. Now, the 48% after 60° centigrade for 10  
22           hours, does that represent the percent of recovery  
23           across the heating step?

24      A.   In this case, it does.

25      Q.   All right. And then if I understand, when you

1           dialyzed the material at a temperature of 5°  
2           centigrade for 13 hours, you got a percent  
3           recovery of 87.2%. Right?

4       A.    Yes.

5       Q.    Meaning, if the numbers are accurate, meaning that  
6           the total loss or the total recovery across the  
7           pasteurization, and now including dialysis, was  
8           87.2% of the starting material, which you had  
9           arbitrarily assigned a percentage of a hundred.  
10          Correct?

11      A.    No. I think one has got to be clear in the  
12           assumption that one makes in it. If one assumes  
13           that all the numbers are reliable, it says that  
14           there is something very strange going on, but it  
15           seems to be coming up. If you assume one of the  
16           numbers are wrong, we need to make an assumption  
17           as to which of the two numbers you intend to make  
18           it. I saw this as an observation not believing  
19           any one of those numbers, since there was no  
20           reason to throw out a particular number just  
21           because it didn't fit my model.

22      Q.    Right.

23      A.    So it continued to trouble me throughout this,  
24           because as I was dialyzing the sucrose out, you  
25           don't get crystal clear material. I was wondering

1 maybe the drop in activity could have been due to  
2 a specific loss of material, just a physical loss  
3 of protein sticking to the cubes, which may not  
4 have related to a loss in activity but purely a  
5 loss of physical matter. So these issues needed  
6 to be resolved.

7 Q. Sure.

8 A. And that was part of the next step, which is the  
9 full issue of scale-up.

10 Q. All right. By the way, how is it that you were  
11 assigned to do these experiments, since there must  
12 have been other people in the company much more  
13 experienced with working with Factor VIII and  
14 Factor IX than you were?

15 Well, I will take out the word "assigned."  
16 How was it that you were doing this work when  
17 there must have been other people in the company  
18 who were more experienced in working with Factor  
19 VIII and Factor IX than you were?

20 A. The issue boils down to what kind of a problem one  
21 is dealing with. The kind of experiments we are  
22 dealing with deals with a hybrid set of issues.  
23 These are issues connected with understanding the  
24 protein per se, the activity per se. There is the  
25 issue of translating that into manufacturing

1 practice, and issues of scale-up. We were doing  
2 it all together..

3 The ideas came to me initially, so I  
4 championed this thing. But again, if it came to  
5 analytical stuff, I'd go to analytical people.  
6 I'd go to John Lundblad or to Milt or Duane.  
7 There were resources I could call for.

8 In fact, when it came to viruses, I would  
9 check with Bob Louie. So the question was I was  
10 receiving evidence, I was presenting material at  
11 clubs, at weekly journal club, not every week.  
12 People were generally aware of it. But that's  
13 where the coin sort of fell.

14 Q. Could you clarify who Bob Louie is?

15 A. Bob Louie was the head of the virology group at  
16 Cutter at that time, if I remember correctly.

17 Q. Right. In fact, it looks to me that on the next  
18 page of your laboratory notebook, page 55, you  
19 actually had a discussion with Bob Louie about  
20 viruses. Right?

21 A. Yeah. I was quite interested at that time. I was  
22 quite interested in moving this thing as rapidly  
23 forward as I could. So I was working on several  
24 fronts, looking at manufacturability, downstream  
25 issues, working with assays, thinking about the

1 next step. One was getting down to addressing  
2 this issue as to why we are pasteurizing it in the  
3 first place. The goal was, in a sense, to reduce  
4 a potential hazard.

5 Q. Okay. What did Bob Louie tell you, if you can  
6 remember, about what you discussed with him on --  
7 is it January 15th?

8 A. 15th or 18th.

9 Q. 15th of '79.

10 A. Again, this is many, many years ago, but I can  
11 tell you in concept the issue was I was trying to  
12 find out what is the best ways to handle some of  
13 this thing? I said, yeah, we could use a  
14 hepatitis B surface antigen, the technique is  
15 available. We could we use viruses. I was  
16 exploring issues from that.

17 And Bob Louie apparently told me at that  
18 time, said, "Yeah, you could try the hepatitis B  
19 surface antigen." In his mind he felt you  
20 wouldn't see any change, and even if you did see a  
21 change in the surface antigen, since that doesn't  
22 correlate with activity, you know. He was quite  
23 neutral on that hepatitis B surface antigen  
24 aspect, but I continued to do the experiment and  
25 learned that it wasn't very much help to me.

1 Q. Did Dr. Louie tell you not to do the experiment?

2 A. No, no. But he sort of had a statement of fact of  
3 his expertise in that area.

4 Q. Okay. Let's continue. You continued to work on  
5 AHF on January 16th, which is page 56. Right?

6 A. Yeah.

7 Q. And then on page 57 you document the hepatitis  
8 antigen study. Correct?

9 A. Right.

10 Q. What were you trying to do here?

11 A. What I did was to spike the hepatitis B surface  
12 antigen into a feed material, pasteurize it, and  
13 try to get an assessment, some handle that I was  
14 searching for to see if I could see a change in  
15 the hepatitis surface antigen.

16 I think there was an assay kit, I don't  
17 remember particularly what, these samples were  
18 handed back to Bob Louie for assessment. The  
19 bottom line in those experiments show that I think  
20 we saw a four-fold drop in the measured level of  
21 hepatitis, the hepatitis B surface antigen.  
22 However, the control sample, which was just a  
23 hepatitis antigen in the excipient buffers, which  
24 I would expect also to show a drop, did not.

25 So again this issue of trying to come to grips

1 with something to build a case on.

2 Q. Right. Now, on January 17th, which again is page  
3 57 of the notebook, you did some experiments with  
4 fibrinogen removal. Is that right?

5 A. Yes.

6 Q. What methods, or what were you thinking about when  
7 you were doing that experiment?

8 A. Again, recognizing this earlier data that  
9 fibrinogen was an issue and I needed to find ways  
10 to do it. Again, discussions within the lab,  
11 essentially with John Lundblad, my supervisor and  
12 others, in this case probably George Mitra, but  
13 the people within our lab group, what can be done  
14 to reduce levels of fibrinogen?

15 An idea came up, use high levels of glycine.  
16 But I did several experiments along this line  
17 trying to look at it. I picked a batch C1051,  
18 which had failed previously, and I ran through the  
19 exercise of trying to reduce the level of  
20 fibrinogen. I'm not too sure this particular --  
21 I'm sorry, 1.6 molar glycine.

22 Q. And?

23 A. And I didn't get convincing results.

24 Q. Now, there was literature, I'm telling you, you  
25 may know this, there was literature, scientific

1 literature having to do with methods of purifying  
2 Factor VIII, including purifying out fibrinogen.  
3 Did you do any research to make yourself familiar  
4 with the literature that existed as of January of  
5 1979? Did you do any research to try and inform  
6 yourself what methods had already been described  
7 in the literature for removing fibrinogen?

8 A. I can't say whether I did or did not, but I'm sure  
9 I must have in the course of my work. Yes, I  
10 probably did refer to whatever was available.

11 I did talk to people within Cutter primarily  
12 to find out which were the best method, because  
13 one of the things that's very apparent in this  
14 game is that to a large extent the effectiveness  
15 of any method depends on your source material,  
16 especially when we're dealing with material at the  
17 front end of the Cohn fractionation process. Nine  
18 percent of the material is usually something else,  
19 so you can get a result somewhere that says yes,  
20 something works, you try to repeat it with your  
21 material, it doesn't work. So my best source of  
22 knowledge was really people within the Cutter  
23 community who was working with raw material  
24 similar to what I was working with.

25 Q. Well, for example, were you aware that glycine had

- 1           been described in the literature as a way of  
2           removing fibrinogen from these solutions?
- 3       A.    I can't recollect. I was probably not sure at  
4           all.
- 5       Q.    Okay. Let's just continue going on with these  
6           experiments. Would you turn to page 61 of your  
7           notebook?
- 8       A.    Yes.
- 9       Q.    What was the purpose? What were you trying to do  
10          in this experiment?
- 11      A.    I think this was another attempt to try to remove  
12          AHF -- I'm sorry, to remove fibrinogen through use  
13          of glycine.
- 14      Q.    Okay. We're talking about page 61 of your  
15          notebook, over onto page 62.
- 16      A.    Yes, onto page 62.
- 17      Q.    Which represents work done on January 29 and  
18          January 30th and onto January 31. Do these  
19          contain the results on January 31?
- 20      A.    They contain the results in terms of my attempt at  
21          using cellulose acetate electrophoresis to measure  
22          the effectiveness of my ability to reduce  
23          fibrinogen. And on page 63, I saw a decrease in  
24          levels of fibrinogen but not significantly enough  
25          to compel me to say yes, I think I've got an

- 1           answer.
- 2       Q.    Would you turn to Page 64 of your notebook?
- 3       A.    Yes.
- 4       Q.    These are assay results, are they not?
- 5       A.    Yes, they are.
- 6       Q.    And what were you learning from the assay work
- 7           that was being done on this material?
- 8       A.    At the bottom of the page, it's reported 60%
- 9           recovery across the pasteurization process.
- 10      Q.    Did these results include the removal of sucrose?
- 11      A.    You know, it's really hard for me to pin it down
- 12           after such a long time, but I think at this point
- 13           I was going through a dialysis step in the
- 14           process, so it probably represented post-removal
- 15           of sucrose.
- 16      Q.    Can you tell me the results that are reported at
- 17           the bottom of lab notebook 64, what the fibrinogen
- 18           content of that material was?
- 19      A.    I believe that refers to the fibrinogen content on
- 20           the previous page, which dealt with it being at, I
- 21           think, 31.8% fibrinogen.
- 22      Q.    Okay. We are going to mark the series of lab
- 23           pages we have been discussing. They are all from
- 24           lab notebook 2021. It's pages 51 through 64 as
- 25           the next exhibits, which includes work done on

1 January 8, January 9, January 10, January 15th,  
2 16th, 17th, 19th, January 23rd, 29th, and January  
3 30th, and January 31 of 1979, and Page 64, which  
4 is February 5th of 1979. I will mark it as the  
5 next exhibit, which is nine.

6

7 MR. JACKSON: Which we've been  
8 discussing. At line 12, --

9

10 **DEPOSITION OF DR. FERNANDES RESUMED**

11 Q. I have asked you off the record to turn to page 75  
12 of your lab notebook, which contains evidence of  
13 work done on March 20th of 1979. Up to this point  
14 in time, had you basically been working with AHF  
15 material that was in inventory for, like scrap  
16 material in inventory, for purposes of doing these  
17 experiments?

18 A. Not really. The initial experiments were from  
19 material in inventory. When I began to look at  
20 the level of fibrinogen, I began using material  
21 from post aluminum hydroxide. This was taking an  
22 aliquot of the manufacturing stream.

23 Q. But material that Cutter was then manufacturing?

24 A. Was then manufacturing.

25 Q. All right. So this was current material that was

1 taken essentially off of the production line. Is  
2 that right?

3 A. Yes, it was.

4 Q. Okay. Then it looked to me, when I was looking at  
5 your notebooks that on page 75 you started doing  
6 work with something called, quote, "Harwell Factor  
7 VIII." Do you see that?

8

9 MR. PRICE: Are you going to mark that?

10 MR. JACKSON: He must not have marked  
11 it. It's not one of the exhibits.

12 MR. PRICE: Okay. All right.

13

14 **DEPOSITION OF DR. FERNANDES RESUMED**

15 A. Yes.

16 Q. Spelled H-A-R-W --

17 A. W-E-L-L.

18 Q. What is Harwell Factor VIII?

19 A. While I was working on this aspect and concerned  
20 about fibrinogen removal, at a very different  
21 level Cutter was also looking at a new piece of  
22 equipment, and there was a piece of equipment  
23 being used in England by the Atomic Energy  
24 Commission, and they had an electrophoresis  
25 apparatus.

1           The difficulties with electrophoresis is that  
2           it creates a lot of heat, so it's only used for  
3           analytical purposes. But apparently at the Atomic  
4           Energy Commission in England they had figured out  
5           a way to remove the heat. It was a rotary kind of  
6           apparatus. And so Cutter was working with them,  
7           and I'm not sure of the details, that wasn't my  
8           part of the project, looking at the use of this  
9           piece of equipment as to way to separate plasma,  
10          just looking at new entities.

11          So when I mentioned it to John in the  
12          January, February time frame, I'm just saying a  
13          problem removing --

14  
15                   MR. GOODELL: "Just having".

16                   MR. PRICE: I'm sorry.

17

18       **DEPOSITION OF DR. FERNANDES RESUMED**

19       A.   -- I'm just having a problem removing the  
20           fibrinogen. John said, "Well, look here. There  
21           is a potential in a few weeks, or maybe a little  
22           while, we are going to get across to this other  
23           unit. Let's see what happens then."

24

25                   MR. GOODELL: Excuse me. I'm sorry.

1 "Access to this other unit".

2 MR. PRICE: I'm sorry.

3 MR. GOODELL: That's all right.

4

5 **DEPOSITION OF DR. FERNANDES RESUMED**

6 A. John said, "Well, look here. There is a potential  
7 in a few weeks, or maybe in a little while, we are  
8 going to get access to this other unit. Let's see  
9 what happens then."

10 So in March a sample of plasma was put  
11 through this electrophoretic unit, and it  
12 separated material into multiple fractions, maybe  
13 15, 20, 25 fractions, and the expert in this area  
14 was George Mitra. He was a coagulation man.

15 So he gave me certain fractions, and he said,  
16 "These are AHF-rich fractions," and so I took  
17 those fractions, they were lyophilized as part of  
18 that process, and I tried to subject it to my  
19 pasteurization experiment.

20 The bottom line on this one is when it came  
21 to look at activities, at the bottom of the page I  
22 noticed that we were still seeing the same kinds  
23 of losses concerned with this.

24

25 MR. JACKSON: Connected.

1                   MR. GOODELL: Connected with this.

2                   MR. PRICE: Connected with this.

3

4           **DEPOSITION OF DR. FERNANDES RESUMED**

5           A.   We were seeing the same kinds of losses connected  
6               with this. This was a difficult experiment only  
7               in the sense that we were dealing with very low  
8               concentrations of material to begin with. So even  
9               though material could have been coming out of  
10              solution, I could not visualize it, because there  
11              was a trace amounts of material.

12                        So we looked at this, quote, "activity  
13                       measurements", close quote. I came away from the  
14                       experiment saying this is not our answer in terms  
15                       of a quick way to remove fibrinogen, and that's  
16                       essentially what's reported on in this page.

17          Q.   Okay. The next page, page 87, and I'm unclear.  
18               Well, the only reason I have 87 is I have got a  
19               date of April 5th, and it's 87. And page 76 is  
20               April 6. Do you know what that means?

21          A.   I'm sorry?

22          Q.   I moved this to the front here because it's got a  
23               date of April 5th. Okay. Good. I will stick it  
24               back in the right spot then.

25

1                   **MR. JACKSON:** Okay. Start with line 22.

2

3           **DEPOSITION OF DR. FERNANDES RESUMED**

4           Q. Okay. You are still doing Factor VIII experiments  
5           on page 76. Is that correct?

6           A. Yeah, I was doing a series of experiments every  
7           time and trying to get a sense of reproducibility,  
8           and I was not succeeding with some of this stuff  
9           to an extent that I would have liked to.

10          Q. Would you turn to page 91 of your notebook,  
11          please?

12          A. Yes.

13          Q. What experiment were you doing on page 91 which  
14          documents work done by you on May 16th, 1979?

15

16                   (counsel reviews exhibit)

17           **MR. GOODELL:** No objection.

18           **MR. PRICE:** What exhibit?

19           **MR. JACKSON:** Plaintiffs' 974.

20           **THE COURT:** 974 is admitted.

21           **MR. JACKSON:** At line 5.

22

23           **DEPOSITION OF DR. FERNANDES RESUMED**

24          Q. What experiment were you doing on page 91 which  
25          documents work being done by you on May 16th of

- 1           1979?
- 2       A.    I want to look at prior pages.
- 3       Q.    Absolutely.  Sure, of course.  It looks to me, and
- 4            you correct me if I'm wrong, but it looks to me
- 5            like you were experimenting with the use of heat
- 6            as a way of removing fibrinogen from AHF.
- 7       A.    I was pausing a little bit to explain the way that
- 8            I had done that experiment, which was essentially
- 9            I knew the activity of AHF in the vial, and in a
- 10          sense I was adding fibrinogen to it and then
- 11          trying to run an experiment to try to remove the
- 12          fibrinogen and see whether I got the same activity
- 13          of AHF that I had gotten initially.
- 14       Q.    What I was asking:  You were trying to remove the
- 15            fibrinogen through the use of heat; is that right?
- 16       A.    Let me read the experiment carefully here.
- 17       Q.    Of course.
- 18       A.    Yes, in a sense I ran it and the material jelled,
- 19            and I tried to centrifuge it and measure the
- 20            activity subsequent to it.
- 21       Q.    Now, were you aware of articles which had appeared
- 22            in the scientific literature that suggested that
- 23            heat may be a way of separating fibrinogen from
- 24            AHF?
- 25       A.    No.

- 1 Q. Were you aware at the time you did this experiment  
2 that fibrinogen precipitates out of solution at a  
3 lower temperature than AHF?
- 4 A. I wasn't aware of it at that time.
- 5 Q. I mean, how do you think you decided to do an  
6 experiment to selectively remove fibrinogen from  
7 AHF using heat?
- 8 A. Because it was based on the general premises at  
9 that time, looking at the fact that fibrinogen --  
10 I knew fibrinogen had actually, at 60°, jelled a  
11 lot more rapidly, and I gained through this  
12 process of incremental understanding it was clear  
13 that at least in my mind saying the concept, if  
14 you remove the jelled portion of the material,  
15 will the rest of it be clear and active.
- 16 Q. Did you surmise when you did this experiment that  
17 it was possible that fibrinogen came out of  
18 solution at a lower temperature than AHF?
- 19 A. No, that wasn't -- I assumed that at 60°  
20 centigrade the material was coming out of  
21 solution. If you ask me whether I knew at lower  
22 temperatures, the answer is no.
- 23 Q. Well, all I am asking you is, were you  
24 experimenting to see whether there was a  
25 difference in precipitation between fibrinogen and

- 1 AHF at certain temperatures?
- 2 A. Yes, as defined by the heading of "selective  
3 denaturation."
- 4 Q. In other words, you were trying to find out  
5 whether you could remove fibrinogen from AHF  
6 preparations by using heat. Correct?
- 7 A. Yes.
- 8 Q. What did you learn?
- 9 A. It's not clear from my results just here.
- 10 Q. Well, it looks like your feed material, in looking  
11 at the middle of the page, had a fibrinogen  
12 content of about 55, and that your treated  
13 material had fibrinogen content of about 22 or 23.  
14 Is that right?
- 15 A. Yes, that's what it says.
- 16 Q. And then the next material tested, it looks like  
17 your feed material had a fibrinogen content of  
18 about 74, and after treatment with heat had a  
19 fibrinogen content of about 36. Is that right?
- 20 A. Yes.
- 21 Q. And did you have the material assayed?
- 22 A. I cannot -- I don't --
- 23 Q. It says sample --
- 24 A. It says samples were submitted for Factor VIII,  
25 see assays, but I am not sure whether I can pin it

- 1 down.
- 2 Q. First of all, are the assay results reported on  
3 page 91?
- 4 A. No.
- 5 Q. Okay. Why don't you then look at subsequent pages  
6 and see if you can identify reports on the assay  
7 results of this material. In other words, whether  
8 you ever got the material back and recorded the  
9 results.
- 10 A. No, this was an experiment, I put in the material  
11 at 8:45 a.m. in the morning. By about 10:50 the  
12 material had jelled, and I removed a sample at  
13 that point of the supernatant.
- 14 Q. All I'm trying to find out is whether you tested  
15 the supernatant for Factor VIII activity.
- 16 A. I gave a sample for an activity measurement. I  
17 don't know at this point what the results were,  
18 but my interpretation, the results were not  
19 remarkable enough for me to pursue that particular  
20 tack.
- 21 Q. Why do you say that?
- 22 A. In terms of subsequent experiments, since I didn't  
23 continue on this particular line.
- 24 Q. Well, is there something in this experiment that  
25 tells you that heat was not an effective way of

1 removing fibrinogen? That we see recorded here.

2 A. It's hard for me to recollect back to the specific  
3 experiment, but hindsight is probably a lot  
4 clearer. But at the time when I was doing those  
5 experiments, the idea of trying to follow a  
6 selective denature based on my -- the information  
7 I had at that point in time didn't indicate to me  
8 that that was the direction to go ahead with.

9 Q. All right. Well, I have a lot more questions to  
10 ask you about some of the details of this, but  
11 we've only got about 10 or 15 more minutes.

12 You have reviewed these laboratory notebooks,  
13 so let me just ask you in general terms to  
14 summarize your work from May of 1979 up until the  
15 time when you left the company. What were you  
16 doing and what kind of progress were you making on  
17 fibrinogen removal and that kind of thing?

18 A. Once we reached the middle of 1979, I began to  
19 sense that I was not seeing good reproducibility  
20 with my reproducibility-type experiments. At that  
21 point in April, I became aware of the fact that  
22 Dr. Robinson had an in vitro type of assay to  
23 measure an enzyme known as DNA-P. DNA, I think,  
24 polymerase associated with the Dane particle.

25 Q. You are talking about hepatitis?

- 1 A. Talking about hepatitis.
- 2 Q. And you are talking about the Dr. Robinson who is
- 3 a virologist at Stanford?
- 4 A. At Stanford University.
- 5 Q. Well respected virologist?
- 6 A. He was recommended to me through Bob Louie.
- 7 Q. Someone at Cutter?
- 8 A. Someone at Cutter.
- 9 Q. Right. Okay.
- 10 A. So again trying to move on parallel lines,
- 11 recognizing that I was going to try initially to
- 12 find the best way to resolve the operational issue
- 13 -- that I needed to get a handle, because I was
- 14 interested, once again, in looking at the
- 15 denaturation kinetics.
- 16 It was very obvious to me that this full
- 17 history of pasteurization at 60° for 10 hours was
- 18 an empirical measurement. All that was done was
- 19 to show that after pasteurization, one could make
- 20 albumin that was noninfectious. Here, because of
- 21 the concern of adding sucrose as well as a mix of
- 22 protein, I was personally interested in getting
- 23 some idea of ratio. I obviously didn't want to
- 24 heat it for 60° for 10 hours, because I realized
- 25 with AHF I was not getting reproducible results.

1           It would have much preferred to heat it for five  
2           hours, but in order to heat it for five hours I  
3           wanted to show myself that that was an adequate  
4           kill to get rid of hepatitis.

5                 So I was searching for an analytical assay,  
6           looked to Dr. Robinson for work, he did a couple  
7           of assays for me. The problem he was having was  
8           that, like in every day life, some of the buffers  
9           in AHF was affecting the assay that he was using,  
10          so I switched to human serum albumin.

11       Q.   Did you complete your experiments on fibrinogen  
12             removal?

13       A.   I think I repeated it once again in August or  
14             September, did another third experiment sometime  
15             towards the end of the year. Again, with unclear  
16             results.

17       Q.   Well, did you make it known to the people within  
18             Cutter that fibrinogen content of these AHF  
19             preparations was critical in terms of being able  
20             to pasteurize those solutions?

21       A.   I think I made it clear. In fact, I do remember  
22             meeting in the middle of the year, sometime in the  
23             June-July thing, when Dr. Duane Schroeder, Milt  
24             Mozen, and myself, and probably John, sat around  
25             and said, you know, maybe we ought to try and find

- 1           some batch of material that's low in fibrinogen  
2           so, you know, I can at least validate the concept  
3           because -- rather than be troubled with  
4           reproducibility issues. So we did have that  
5           discussion, and I think they did recognize this  
6           aspect.
- 7       Q.   Do you think that those, the two scientists that  
8           you mentioned, did you have the sense that they  
9           agreed with you that it was likely that the  
10          fibrinogen content of the material was an  
11          important element of whether or not AHF  
12          preparations could be pasteurized?
- 13      A.   I came away from the meeting recognizing that they  
14          understood the problem.
- 15      Q.   In fact, when you filed your patent application,  
16          or a patent application was filed with your name  
17          on it as the inventor, that patent disclosed that  
18          fibrinogen content was a crucial element of  
19          pasteurizing AHF, did it not?
- 20      A.   I'm not sure which document you are referring to  
21          specifically.
- 22      Q.   Well, did you file a -- or do you know that a  
23          patent application was filed in April of 1980 for  
24          the invention that was first disclosed in December  
25          of 1978?

1 A. Yes. And I do know that prior to leaving Cutter I  
2 remember writing a memo to the patent attorney  
3 reminding him that after our experiments that  
4 fibrinogen wasn't working out that maybe it ought  
5 to be removed from any further concern.

6 Q. But do you know that the application that was  
7 filed with your name on it as the inventor  
8 included statements in there that documented the  
9 importance of fibrinogen removal in terms of the  
10 pasteurization process?

11 A. I cannot recollect the exact statement. If I see  
12 that document, I can give you an opinion on it.

13 Q. I have it here, and I am going to mark it in a  
14 minute, but before I do that I want to mark the  
15 next exhibit. First of all, let's mark the  
16 Selective Denaturation of Fibrinogen and AHF by  
17 Heat. Let's mark that page of the laboratory  
18 notebook. Page 91 of book 2021. Right. Which  
19 indicates work done on May 16th of 1979 as the  
20 next exhibit, which would be Exhibit 10.

21

22 MR. JACKSON: We've already put it in.

23 MR. PRICE: That's exhibit what number?

24 MR. JACKSON: That was Exhibit 10, and  
25 he's also offered Exhibit 11.

1 DEPOSITION OF DR. FERNANDES RESUMED

2 Q. Here is Exhibit 11, which is a memo from you, it  
3 looks like, to J. Giblin. Is that correct?

4 A. Yes.

5

6 MR. JACKSON: So Plaintiffs would offer  
7 11, which is the memo, at this time. It's  
8 Plaintiffs' 975.

9 MR. GOODELL: No objection.

10 THE COURT: 975 is in. This is a memo  
11 from the witness to J. Giblin?

12 MR. JACKSON: Yes.

13 THE COURT: 975 is admitted.

14

15 DEPOSITION OF DR. FERNANDES RESUMED

16 Q. Dated October 11th, 1979, entitled Protein  
17 Stabilization During Pasteurization. Do you see  
18 that?

19

20 MR. GOODELL: Counsel, excuse me for one  
21 second. I don't know if the copy you gave  
22 includes a fax cover sheet which probably  
23 should not be part of the exhibit.

24 MR. JACKSON: Is the last page a fax  
25 cover?

1                   **MR. PRICE:** Okay. You want to show that  
2                   one?

3                   **MR. JACKSON:** Yeah.  
4

5       **DEPOSITION OF DR. FERNANDES RESUMED**

6       Q. With copies to Bradley Mozen, Victor Cabasso and  
7       John Lundblad. Do you see that?

8       A. Yes.

9       Q. Did you prepare this memo?

10      A. Yes, I wrote it.

11      Q. Did this memo reflect the state of your knowledge  
12      at the time you prepared it with respect to the  
13      subject matter in the memo?

14      A. Yes, it did.

15      Q. Do you see on Page 2 of the memo the reference to  
16      Seeger's work?

17      A. Yes, sir.

18      Q. And now an actual citation for that work?

19      A. Yes, I do.

20      Q. It says, quote, "Emphasis was placed on sucrose  
21      based on Seeger's comment in 'Purified Prothrombin  
22      & Thrombin,'" and then it gives the citation and  
23      title of the article. Does that mean that you  
24      selected sucrose because of your knowledge of the  
25      Seeger's article?

- 1 Q. No.
- 2 A. What do you mean, quote, "Emphasis was placed on  
3 sucrose based on Seeger's comment," end of quote?  
4 What does that mean?
- 5 A. It meant that after I had done the initial  
6 experiments and showed that I had a system work,  
7 working, I was personally comforted by the fact  
8 that another individual had used sucrose, also,  
9 and I continued on using sucrose.
- 10 Q. Why did you place emphasis on sucrose because of  
11 Seeger's work?
- 12 A. I'm not too sure. Thinking back to my state of  
13 mind in 1979, I'm not too sure specifically what  
14 the point I was trying to make. But from then I  
15 began looking at sucrose potentially from a  
16 manufacturing standpoint as being, oh, probably  
17 better than anything else or as good, because I  
18 had done work on maltose earlier with equivalent  
19 results.
- 20 Q. Let me ask you this: Do you think that the  
21 emphasis that you placed on sucrose was based at  
22 least in part on your knowledge of the work that  
23 Seegers had done?
- 24 A. Once I saw the document, it was part of my  
25 knowledge base. There were a couple of things

1           that came into it. One, yes, there is another  
2           document that shows that sucrose can be used, but  
3           it was also saying that, you know, let's be  
4           careful about talking about all carbohydrates  
5           being a possibility, because in earlier  
6           disclosures, I had sort of painted a pretty broad  
7           range.

8                     Here is an article also that says look at --  
9           it doesn't work on all proteins. It's that  
10          combination that led me to include that as part of  
11          this document.

12       Q.    Include the Seeger's article?

13       A.    Yes.

14       Q.    Bear with me just a minute. I'm confused about  
15          one thing, and maybe you can help me. If I took  
16          some more time, I could find the document that I'm  
17          looking for, but we're up against it here in terms  
18          of the time we've allotted.

19                     Is this Exhibit Number 12 the patent that was  
20          granted on April 3, 1984 with you named as one of  
21          the inventors entitled, Pasteurized  
22          Therapeutically Active Protein Compositions?

23

24                     **MR. JACKSON:** And the patent is already  
25          in evidence, Your Honor, as Plaintiffs' 736,

1 which is identified as Plaintiffs' 12 in the  
2 deposition.

3 Okay. Go ahead at line 20.

4

5 **DEPOSITION OF DR. FERNANDES RESUMED**

6 A. Yes. If I could make a statement that I left the  
7 company in April of 1980 and was not an active  
8 participant in whatever happened.

9 Q. I understand. Let's mark this as the next  
10 exhibit.

11

12 **MR. JACKSON:** Which is Plaintiffs' 361.  
13 It's an abstract of disclosure from Drs.  
14 Fernandes and Lundblad, and we'd offer into  
15 evidence as Plaintiff's 361.

16 **THE COURT:** What is it exactly? I'm  
17 sorry.

18 **MR. JACKSON:** It is an asbtract of a  
19 disclosure --

20 **THE COURT:** Okay.

21 **MR. JACKSON:** -- application of the  
22 United States patent.

23 **THE COURT:** Any objection?

24 **MR. GOODELL:** No.

25 **THE COURT:** 361 is admitted.

1 DEPOSITION OF DR. FERNANDES RESUMED

2 Q. My question is that it appears, and I have marked  
3 as Exhibit 13 --

4  
5 MR. JACKSON: Plaintiffs' 361 here.

6  
7 DEPOSITION OF DR. FERNANDES RESUMED

8 Q. -- an abstract of a patent disclosure. It's got a  
9 date at the top, and I don't know how it got  
10 there, of October 31, 1980, but it indicates an  
11 original patent filing of March 5, 1980.

12 What happened to the, if you know, to the patent  
13 that was filed in your name in March of 1980, or  
14 in the year 1980, for that matter? Because the  
15 patent that was created on April 3, 1984 indicates  
16 a filing date of December 20, 1982.

17 A. This is something I'd have to defer to the patent  
18 people. I have no clue.

19 Q. Well, the disclosure is a modification or a  
20 continuation, that is what we marked as --

21  
22 MR. JACKSON: 361 here.

23  
24 DEPOSITION OF DR. FERNANDES RESUMED

25 Q. -- of a document filed on March 5, 1980. Do you

1 think you saw a draft of Exhibit 361 before you  
2 left the company?

3 A. I may or may not have seen a draft. I don't  
4 recollect.

5 Q. Do you know whether you saw the draft of the  
6 original patent application before you left, which  
7 apparently was filed on March 5, 1980?

8  
9 **MR. PRICE:** You want to put that one up  
10 so they can see it?

11 (Exhibit displayed on ELMO)

12

13 **DEPOSITION OF DR. FERNANDES RESUMED**

14 A. I may have seen drafts of it.

15 Q. Well, when you left the company in the spring of  
16 1980, did you believe or had you come to the  
17 conclusion that fibrinogen content of these AHF  
18 preparations was a critical element of  
19 pasteurization?

20 A. I had come to the conclusion that I saw an  
21 opportunity in terms of pasteurization. I came at  
22 the end of 1979. My mental state was that I  
23 basically dabbled in each one of these areas. I  
24 had shown the concept of reducing opalescence  
25 during pasteurization.

1 I personally was not comfortable with some of  
2 the quantitation that had been done, whether it  
3 was in terms of activity or not. I wasn't  
4 comfortable in terms of the hepatitis or the DNA  
5 polymerase.

6 I began to question my own ability in some of  
7 these -- I knew it looked like there was a mix of  
8 proteins there, there was something that had to be  
9 worked up. Fibrinogen in my mind seemed -- that  
10 was something that I knew played a role. If you  
11 asked me should I remove that fibrinogen down to  
12 0%, 10%, 15%, I could not have told you.

13 Q. But you did come to a conclusion that the  
14 fibrinogen content played a role?

15 A. I came to the conclusion that the current mixture  
16 that we were working, which considered all of  
17 these components, was not adequate and some  
18 optimization had to be done, something had to be  
19 played around with it.

20 Q. Thank you.

21

22 THE COURT: Is that it?

23 MR. JACKSON: That's it, Dr. Fernandes.

24 THE COURT: Okay. We'll take a short  
25 break before we have the next witness.

1 WHICH WAS ALL OF THE TESTIMONY GIVEN BY  
2 PETER FERNANDES ON SAID DATES  
3  
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1       STATE OF INDIANA       )       IN THE MARION SUPERIOR COURT  
2       COUNTY OF MARION     ) SS: CIVIL DIVISION       ROOM ONE  
                                  ) CAUSE NUMBER       GRO-C

3  
4       GRO-A SR., and GRO-A personally       )  
          and as surviving guardians of       )  
5       GRO-A JR., Deceased,                )  
  )  
6                               Plaintiffs,        )  
  )  
7       vs.                                        )  
  )  
8       ARMOUR PHARMACEUTICAL CORP.,        )  
         CUTTER LABORATORIES,                )  
9       DIVISION OF MILES, INC.,            )  
         BAXTER HEALTHCARE CORPORATION,       )  
10       f/k/a Hyland Therapeutics,        )  
         Division of Travenol                )  
11       Laboratories, Inc., and            )  
         ALPHA THERAPEUTICS CORPORATION,       )  
12    )  
                             Defendants.        )

13  
14  
15                               REPORTER'S CERTIFICATE

16  
17               I, Mary Beth Hall, Reporter of the Superior Court  
18       of Marion County, Civil Division, Room One, State of  
19       Indiana, do hereby certify that I am the Official Court  
20       Reporter of said Court, duly appointed and sworn to  
21       report the evidence of causes tried therein.

22  
23               That upon the hearing of this cause on the 12th  
24       and 13th days of February, 1997, I took down, by  
25       machine recording, all of the statements by counsel,  
26       the evidence given during the trial of this cause, the

1 objections of counsel thereto, and the rulings of the  
2 Court upon such objections, the introduction of  
3 exhibits, the objections thereto, and the Court's  
4 rulings thereon.

5

6 I further certify that the foregoing transcript,  
7 as prepared, is full, true, correct and complete as to  
8 the testimony given by Peter M. Fernandes.

9

10 IN WITNESS THEREOF, I have hereunto set my hand  
11 and affixed my Official Seal this \_\_\_\_\_ day of  
12 \_\_\_\_\_, 1998.

13

14

Mary Beth Hall, Official Reporter  
Marion County Superior Court  
Civil Division, Room One

15

16

17

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25

26

Pete M. Fernandes, PhD  
 GRO-C  
 GRO-C California GRO-C  
 Work: GRO-C  
 Home: GRO-C

1/92 to Present Chiron Corporation, Emeryville, CA 94608

Experience:

1/96 - Present: Director, Process Development Planning, Chiron Corporation

- \* Responsible for strategic planning for process development activities across multiple domestic / international Chiron sites.

12/91 to 1/96: Vice President, Process Development, Chiron Corporation

- \* Built an integrated Process Development Division consisting of 85 technical staff with expertise in the design, production and characterization of Yeast, Mammalian and E.coli derived products and with capabilities in formulation and drug delivery for Vaccines and Therapeutics applications.

Divisional responsibilities include process design, PLA quality process/product characterization, formulation / drug delivery and clinical production for Phase I/II trials.

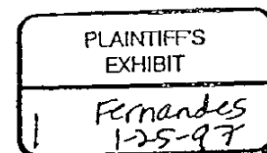
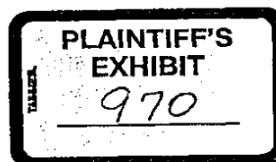
5/80 to 12/91 Cetus Corporation, Emeryville, CA 94608  
 [In December 1991, Cetus was acquired by Chiron Corporation]

Experience

6/91 to 12/91 Vice-President, Process Development  
 5/90 to 5/91 Senior Director, Development Division

- \* Participated in European (CPMP) and US (FDA) Regulatory review for licensure of Proleukin IL2.

- \* Worked with Chiron Corporation management, prior to the merger, to develop/implement plans for successful integration of Development and Manufacturing divisions.



4/89 to 4/90	Senior Director, Process and Product Development
7/85 to 3/89	Director, Process and Product Development
5/80 to 6/85	Scientist

Since 1980 I have been involved at levels of increasing responsibility dealing with the development of GMP processes for the production of therapeutic proteins as well as the design and characterization of protein formulations for therapeutic applications. Key programs included, Beta-Interferon, Interleukin2, Macrophage Colony Stimulating factor, Immunotoxins, Human Monoclonal Antibodies and retroviral vector production.

\* Managed a staff of upto 45 people ( including 14 PhD Scientists) across three broad disciplines:

*Microbial Fermentation and Cell Culture Operations:*

Recruited and established strong in-house capabilities in cell culture scale-up, medium development, recovery operations, GMP production.

*Purification Development:*

Developed, characterized and validated manufacturing processes for over 10 therapeutic proteins.

*Formulation and Pharmaceutical Sciences:*

Recruited scientists with specific experience in protein modification, controlled delivery, clean room operations, lyophilization technology, etc

Significant projects where I have had primary technical responsibility have included:

*Group Leader, Development - Interleukin-2*

June 1984-Dec1991

\* Participated in defense of IL2 dossier for approval by CPMP (Europe) and FDA

\* Identification, coordination of multiple R&D tasks required to complete Manufacturing and QA section for filing of Proleukin IL2 with CPMP/FDA

\* Development of a commercial production process and formulation to support clinical development of product.

\* Development of processes / formulations for PEG-modified Interleukin2.

*Group Leader , Tumor Necrosis Factor*

July 1985 - May 1987

\* Directed all technical activities related to filing INDs and completion of Phase I Trials

1-2

Group Leader , Beta Interferon

March 1983 - May 1987 <sup>3</sup>

- \* Managed post-INDA process scale-up to 1000L microbial fermentation and downstream operations.
- \* Co-developed Beta-Interferon purification process. Because of the hydrophobic nature of this protein, a proprietary formulation process consisting of high pH treatment of Beta-Interferon and Human serum albumin was developed.

Scientist , Fructose Project

May 1980 - February 1983

- \* Developed a pilot scale process for production of Fructose from glucose using a coupled multi-enzyme fluidized bed reactor system. The process involved immobilization of Pyranose 2-Oxidase/Catalase on industrial resins. Project was terminated for business reasons.

6/76 to 4/80      Cutter Laboratories ( Now Bayer ) , Berkeley, California 94508

Experience:

6/76 to 4/80      Senior Biochemical Engineer

- \* Specific contributions included :  
The development and scale-up of a process to produce a chemically modified human-derived immunoglobulin product. A proprietary formulation was developed to stabilize against protein aggregation. This product, 'GAMIMUNE'-IVIG was approved by FDA in 1980.

The development of several proprietary protocols for pasteurization of proteins from human plasma .

1/75 to 5/76      Amour Pharmaceutical Company, Kankakee, Illinois 60808

Experience:

1/75 to 5/76      Senior Chemical Engineer

- \* Trouble-shoot purification processes for Anti-hemophilic Factor from Human derived plasma.
- \* Evaluated feasibility of large scale continuous purification process for extraction of multiple proteins from human plasma via the Cohn cold ethanol process.

1-3

Academic Background:

Ph.D. Chemical and Biochemical Engineering, 1975  
Rutgers University, New Brunswick, New Jersey

B.S. Chemical Engineering, 1970  
Indian Institute of Technology, India

Bibliography:Patents:

- \* Formulation for Lipophilic IL2 Proteins, U.S. Patent # 4,992,271 (1991).
- \* Process for Recovery and Formulation of Interleukin 2, U.S. Patent # 4,604,377 (1986).
- \* Method and Reagent for Pyranosone Production, U.S. Patent # 4,569,910 (1986).
- \* Pasteurized Therapeutically Active Protein Compositions, U.S. Patent # 4,440,679 (1984).
- \* Process for Recovery and Formulation of Interferon Peptides, U.S. Patent, # 4,462,940 (1984).
- \* Stabilization (Formulation) of Intravenous Gamma Globulin, U.S. Patent # 4,186,192 (1980)

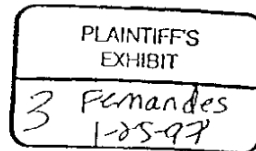
Publications:

- \* The Production and Testing of Recombinant Human Interferon Beta, Joint IABS / WHO Symposium on Standardization and Control of Biologicals produced by rDNA Technology, Geneva, Switzerland; Development of Biological Standards, Vol. 1, 69 (1983).
- \* Preparation of a Stable Intravenous Gamma-Globulin, Vox Sanguinis, Vol. 39, 101 (1980).
- \* Modeling and Process Optimization of Multi-Enzyme Reactor Systems, Chemical Technology, July 1975.
- \* Characterization of Glucose Oxidase Immobilized on Collagen, Molecular and Cellular Biochemistry, Vol. 1, 127 (1973).

Recent Courses:

- \* Financial Analysis for Non-Financial Executives, 1995. Haas School of Business, UC Berkeley

1-4



3-1

TO: Bart Bradley

FROM: Liter Fernandes / John D. Kennedy - 2-1  
Inventor Date

SUBJECT: Disclosure  
(Conception of Invention)

WITNESS: GRO-C 10/25/78  
Read L Date

TITLE: Pasteurization of Albumin and  
Immune Serum Globulin in the presence  
of Carbohydrates.

WITNESS: GRO-C 10/25/78  
Read L Date

REMEMBER: Whenever you are faced with a problem and you have an idea of how to solve it, that is the time to disclose it - not after the invention is complete, SO IT IS BY tomorrow, someone else may beat you to it.

\*\*\*\*\*

Disclosure MUST BE IN INK and is best made by following the consecutive steps listed below

- |  |            |  |
|--|------------|--|
| 1. Date  |            | 5. Drawing   |
| 2. Title                                       | Mechanical | 6. What drawing shows  |
| 3. Problem                                     |            | 7. How it works  |
| 4. Your solution, compared to current practice |            | 8. Your signature and two witnesses who understand your disclosure |

DISCLOSURE:

Pasteurization of Defatted Albumin and Immune Serum Globulin in the presence of carbohydrates. 10-25-78

In the Lohr process, the susceptibility of hepatitis contamination in albumin, for example, is effectively minimized by pasteurization (60°C, 10 hours) in the presence of various additives like sodium caprylate, sodium acetyl tryptophan, etc. These materials bind to albumin and stabilize the whole protein against thermal denaturation.

Gamma globulin, unfortunately, is in its current formulation is unstable at 60°C and gels within the 10 hour incubation period. The Lohr process, however, is unique in the sense that to date no contamination of the product with hepatitis has been detected. Other processes available to produce ISG are always suspect in terms of its ability to transmit hepatitis. This fact alone has hindered technically more efficient processes from being exploited on a large scale.

Quite surprisingly, we have shown that in the presence of certain carbohydrates, gamma globulin loss can be prevented from gelling during the pasteurization process.

NOTE: Use both sides of page if necessary

111 4370

3-2

We have evidence to show that 5% MISC in 10% dextrose can be pasteurized and appears only slightly more opalescent. The physical properties, density, viscosity do not appear to have been altered.

This material could be considered as final container material, but if although if further clarity is required the material can be filtered and refilled in a hepatitis free environment.

Although data on the level of aggregation, presence of new antigenic sites, AC activity, antibody levels, etc need to be evaluated, the possibility of a therapeutically effective heat pasteurized gamma globulin appears optimistic. The heating process will also denature any proteolytic enzymes present in the product.

In the manufacture of defatted albumin, pasteurization in the presence of sodium caprylate is not appropriate since its presence does not result in a fat free product. The use of carbohydrate here, ~~is a distinct possibility~~ to stabilize the protein is a distinct possibility.

It should be noted that all carbohydrates could theoretically be used to stabilize these plasma proteins.

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PLAINTIFF'S  
EXHIBIT

1000

CUTTER Laboratories, Inc.

Page No.

37

Record Book No. 2021

Continued from Page

### Pasteurization of 52 MISG

3x50 vials of 2021-04 (52 MISG in 10% Dextran) were pasteurized at 60°C for 15 hours. At the conclusion of pasteurization, visual observation of the contents indicated no gelling. The material was slightly more opalescent than the untreated material.

The contents were filtered through a Whatman 54 followed by a 0.45µ / 0.12µ absolute filter.

Apparently, this heating step can be utilized to suppress an NVG or 1965 product against hepatitis contamination.

TSSO untreated material	97.5%
TSSO, 60°C, 15 hours	95.3%

PLAINTIFF'S  
EXHIBIT

4 Fernandes  
6-25-97

Read & Understood

Gracie L. S. G.

10/25/78

I affirm that the above is a true record of work done by me

GRO-C

Continued on Page

Date

10-25-78

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MIL 028888

# CUTTER Laboratories, Inc.

38

Research Record Book No. 2021  
Continued from Page 37

Page No.

Pasteurization expts (Continued)

60°C, 10 hrs

PK 2631  
2634 - 15% MISC  
Inulin  
0.5M dextrose  
5% MISC

jellied  
jellied  
jellied  
clear

2021-35 H  
pasteurized &  
filtered 2661

PK 1709 maltose 94% 1.1%

Milky → on filtration (v. difficult)

PK 2661 maltose 94%

clear → on filtration less milky & more clear

Although samples 2661 & 0.5M dextrose were clear on heating some particles were still evident.

5% Albumin (untreated) jellied  
5% Albumin, 10% dextrose v. clear  
10% maltose less clear.

① Glycine buffer contributes to turbidity on heating  
② Glucose better than maltose

will try selective alk denaturation → filtration to remove unstable material and further heat treatment (60°C, 10 hrs)

PK 2661 heated for 3 hrs @ 60°C. Good filtered, refilled & pasteurized.

2.20 ml 3 hrs 60°C - filtered, 5°C overnight, pasteurized  
1 x 5 ml - filtered, pasteurized immediately  
1 x 5 ml 2021-24 - RSG defatted albumin in 10% maltose

I affirm that the above is a true record of work done by me

GRO-C

JUL 2 1978

Continued on Page

Date

10/27/78

DAY

PLAINTIFF'S EXHIBIT

1001

PLAINTIFFS EXHIBIT

5 Fernandes 1-2597

5-1

MIL 028889

# CUTTER Laboratories, Inc.

Page No. 39

Research Record Book No. 2021-~~off~~

Continued from Page X

## Polio & Measles Potency in Source Plasma

BM <del>all</del> <del>over</del>	Polio Type		Measles	
	Standard	Potency ratio	Standard	Potency ratio
8M 7266	1:1600	.07	1:283	0.2
7264	1:1600	.05	1:283	0.2
7269	1:1600	.06	1:400	0.1
7267	1:1600	0.07	1:400	0.1
7268	1:1600	0.1	1:400	0.1
7270	1:1600	0.06		
7271	1:1600	0.07		
7272	1:1600	0.10		
	ave .0725		ave	14

ave. for 5-9 MSG

0.32

0.50

Assuming plasma contains  
large (0.9 - 1.5 gm%)

1.2 gm % Ig G

Plasma

MISG

Equivalent 5719G

Measles  
Potency

$$\frac{0.14 \times 5}{1.2} = 0.58$$

$$\frac{0.07 \times 5}{1.2} = 0.29$$

0.50  
0.32

I affirm that the above is a true record of work done by me

GRO-C

Continued on Page

Date

11/1/78

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INT-503

5-2

MIL 028890

# CUTTER Laboratories, Inc.

Research Record Book No. 2021

Continued from Page \_\_\_\_\_

Page No. 41

## AA Analysis

### Samples Submitted

2631  
2634  
2649  
2660  
2661  
2662  
2664  
2677  
262704  
2705  
2709

### Additional Lts from same batch

2632, 2633

2663

2680

2679

2710

2703

## Effect of Casylate, tryptophan on MISA pasteurization

### Sample composition

5% MISA in .004M Casylate  
 .004M acetyld tryptophan  
 .004M Cas + .004M trypt  
 .004M Cas + 10% Maltose  
 .004M trypt + 10% Maltose  
 10% Maltose

All  
gelled solid

→ slightly gelatinous

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Date

11/7/78

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100ml of 5% MISO and added ~58 gm of sucrose  
Total volume ~ 158 ml  
Protein ~ 3.33%

The material was pasteurized 10hr, 60°C and  
dialyzed against WFI. Following this treatment  
the material was concentrated and formulated  
to 10% Maltose 0.1M glycine

Absolute viscosity / poise of final preparation  
= 213.5 cP  
= 1.867 cP

From 1985-83, Abs. viscosity of 10% Maltose, 0.1M Gly, 5% MISO  
= 1.81685 cP

2 sucrose 2.78 cP

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Protein Content of desalted MISC = 6.29%

81 ml of above + 19 ml WFI = 5.1% (100 ml)

- ① low salt MISC 50 ml
- ② in 5% Maltose 100 ml
- ③ 10% Maltose 50 ml
- ④ + 1 HCl 50 ml
- ⑤ in 15% Maltose 100 ml
- ⑥ in 50% Maltose 100 ml
- ⑦ in 0.3 M Cl<sub>2</sub>/0.45% Li 150 ml

12/7/78

230 ml of 6% MISC (low salt)

175

Added 175 mgms sucrose

Final volume 300 ml 325 ml

Sucrose conc? 5.5%

Pasteurized for 10 hrs at 60°C - particles

Protein conc? 1.16%

Dialyzed against WFI over weekend. Slight pptn.

Concentrated using XM-100A to 6% added Maltose  
& glycerol. Stride filtered  
Filled 16 x 10 ml

See pg 46 for results on heat pasteurized MISC  
2021-43

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2021-44

Final Bulk Samples on Rabies Virus - see  
PL 2716

Pasteurization of AHF

2 x 30ml vials of AHF (MS138) were reconstituted  
according to label directions in 10ml WFI ea.

The contents of one of the vials was autoclaved  
with sucrose (800mg) and both vials were  
incubated @ 60°C for 13 hours.

- Visual observation of the two vials showed
- ① visual clarity in the vial containing sucrose
  - ② opalescence in vial not containing sucrose

Opinion: Entirely product sucrose is able to protect the AHF  
from turning opalescent.

Witness:

James J. Lugo

4/8/79

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EXHIBIT  
7 Fernandes  
1-25-97

7-1

TO: Albert Bradley

FROM:

GRO-C

SUBJECT: Disclosure  
(Conception or invention)

WITNESS:

GRO-C

12/10/78  
Date

Read & Understood

WITNESS:

GRO-C

12/14/78  
Date

Read & Understood

TITLE: Pasteurization of Fibrinogen, Factor VIII, Factor IX (Kongie), Gamma Globulin, & Plasma  
C1a and other plasma proteins in the  
presence of carbohydrates.

REMEMBER: Whenever you are faced with a problem and you have an idea of how to solve it, that is the time to disclose it - not after the invention is complete, NO !!! By tomorrow, someone else may beat you to it.  
\*\*\*\*\*

Disclosure MUST BE IN INK and is best made by following the consecutive steps listed below:

1. Date
2. Title
3. Problem
4. Your solution, compared to current practice

Mechanical

5. Drawing
6. That drawing shows
7. How it works
8. Your signature and two witnesses who understand your disclosure

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DISCLOSURE:

12/14/78

This disclosure is for a wider application than the one entitled "Pasteurization of defatted albumin and human serum albumin in the presence of carbohydrates" submitted on 10/25/78.

The problem addressed in this disclosure is primarily directed at inactivating the Hepatitis virus by heat pasteurization (65°C, 10 hours). The presence of the infective particles in final containers Fibrinogen, AHF, & Kongie severely limits the usefulness of these products. For gamma globulin this is not a problem when it is manufactured by the Cohn process. However, it limits us in the search for more efficient processing methods for it has been shown that non-Cohn processed gamma globulin is capable of transmitting hepatitis.

With our present processes, Fibrinogen, Factor VIII, Factor IX, Gamma globulin can't be pasteurized because they are very heat labile. Even though the source plasma is tested for hepatitis by radio-labeled sensitive methods the test is not sensitive enough to detect trace levels of the virus. When the plasma pool is contaminated, the hepatitis virus is usually concentrated in Factor IX (Kongie), Factor VIII (AHF), Factor I (Fibrinogen) and Factor II (Plasminogen).

Quite surprisingly we have shown that in the presence of certain carbohydrates there it may be possible to protect these

7-2

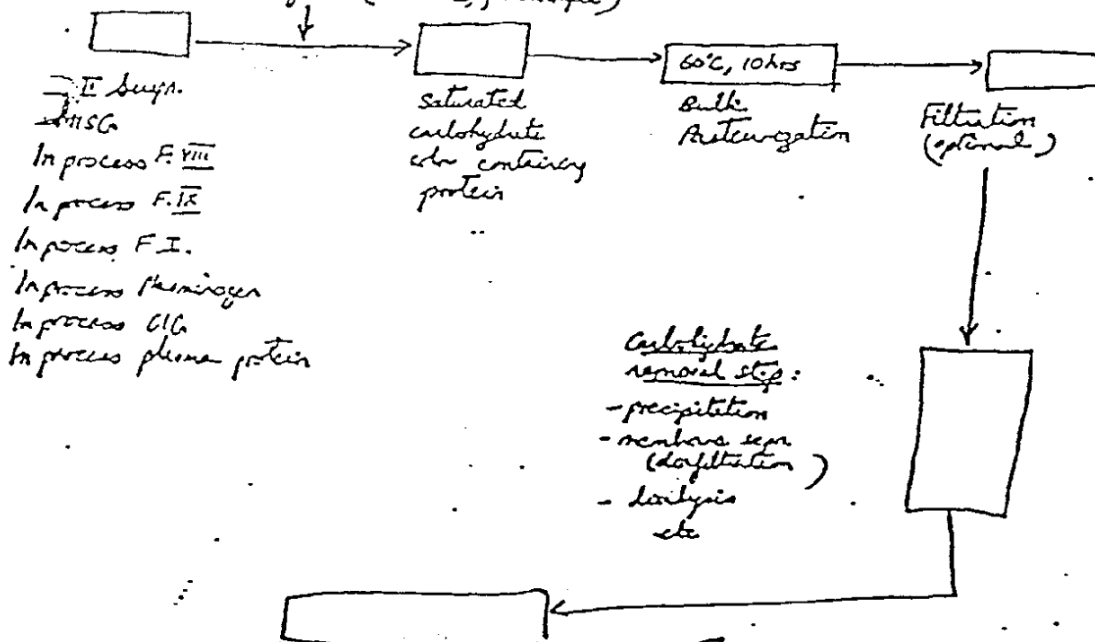
fragile proteins during pasteurization

These sugars are then to be removed after the pasteurization step and should not be present in the final container product.

We are in the process of subjecting these proteins to pasteurization and characterizing the final product in terms of biological activity and physical appearance.

The key to the protective effect, is to pasteurize in the presence of saturated carbohydrate. The literature makes one reference to this fact in the paper by Seegers (attached) who was able to show only a  $3\frac{1}{2}\%$  loss in activity of ~~prothrombin~~ Thrombin, due to heating to  $50^{\circ}\text{C}$  for 48 hours. The paper is

A schematic of our proposed process is shown below:



Since this is a bulk pasteurization step, all processing on the "reaction" side would have to be done in a hepatitis free area. Should be noted that all carbohydrates could theoretically be used to protect the protein during pasteurization

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Continued from Page

Using C-1051 material:

A 10ml + 3g  
B + 5g  
C + 10g  
D + 0g  
E Kynge by self

8:15am

inserted A B C D

8:25am

inserted E

6:40pm ← Collected →

Results on sucrose fastenized MISG - Final formulation

5% MISG, 10% maltose, 0.1M glycine

Exptl details 2021-43		2.0mm		2.0mm	
GPC	2.0ml	2.0mm	2.0mm	2.0mm	2.0mm
	6.7%	7.4%	75.1%	0.7%	
SISPE	H <sub>2</sub> L <sub>2</sub>	H <sub>2</sub> L	H <sub>2</sub>	HL	H
①	8	7.6	9.6	11.9	36.7
②	6.5	7.7	10.1	11.2	38.3
					26.3
					25.8

Antibody potency assays:

Fastenized MISG

Monoclonal

Diphtheria (units/ml)

0.3

0.8

>1<2

AC

8.4mg/CHSD

Past. MISG

1948-98

Control

1948-99

Past.

passed safety

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## Pasteurization studies on Korte

A vial of M 5368 Korte, lyophilized were reconstituted in 10 ml each with WFI

Vial #	Sucrose added, g	60°C/22 1/2 hours	Post <del>At</del> T <sub>580</sub> 2 in sucrose
1	3	clear	95.218
2	5	clear	97.04
3	8	clear	98.216
4	10	clear	97.044

## Kortine

1 vial reconstituted in 20 ml and divided into two aliquots

Vial #	Sucrose added, g	60°C/22 hours	Post T <sub>580</sub> 2
5	5	clear	97.04 2
6	10	clear	98.21 7

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## Pasteurization of ISG

150 gm R.I., 84 7213 resuspended in WFI Total volume ~ 500 ml.

Filtered 500 ml thru 0.8  $\mu$  Nalgene. Protein 9.712.

Added 200g. sucrose to 250 ml of ISG solution. Total volume after sugar addition 365 ml.

Pasteurized 10 hrs @ 60°C

## Pasteurization of AHF

5 vials of AHF M 3094, AHF units 243 (alcl) 0.13 gm protein

Exp. Dec 75 reconstituted in 10 ml WFI ex.

Total volume 50 ml.

Added 40 gm sucrose.

Pasteurized 10 hrs @ 60°C

## Removal of sucrose ISG

The pasteurized material in dialyzed against WFI for 24 hr

precipitated material present in dialysis bags.

Probable reasons: (1) alcohol in R.I. parts  
(2) low salt conditions  
- precipitation of cryoglobulins.  
Discontinued run.

## AHF

The pasteurized material was dialyzed against f.c. AHF buffer for 12 hrs.

0.3M Glycine

0.15M NaCl

0.01M Sod citrate  $\cdot 2H_2O$  (FW 294)

~ 50 ml in dialysis bag in 20L of above buffer.

Initial feed A<sub>280</sub> (bottle) A<sub>280</sub> = 0.7 x 25

A<sub>280</sub> in dialyzed material = 0.33 x 25

Vol of dialyzed material ~ 200 ml

(0.84 x 2)

221-48A

221-48B

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## Coculation results

Feed 27 units/ml at  $A_{280}$  0.7425 = 17.5 units

Pasteurized Material 200-488 2.5 units/ml @  $A_{280}$  0.16 x 25 = 4 units

Feed 27 / 17.5 = 1.54 units/ $A_{280}$

Pasteurized material 2.8 / 4 = 0.7 units/ $A_{280}$

= 45.4% recovery.

## Pasteurization of Defatted Albumin

2021-49

62.5 gm of Albumin (acetone dried powder) suspended in 624 0.15M NaCl. 31.75 gm of WFI washed activated charcoal added (washed on Whatman #54).

Initial pH 5.0. Adjusted pH down to 3.0 with 6N HCl and stirred for 1 hour. Centrifuged and filtered thru Whatman #54, 0.45  $\mu$ , 0.2  $\mu$ . (Filtered only 200 ml). Raised pH to 7.04 and froze in 6N HCl.

-40°C freezer.

1/8/79 Sampled 4 x 10 ml of above soln.

Portion #1 10 ml + 2g sucrose  
#2 10 ml + 4g sucrose  
#3 10 ml + 6g sucrose  
#4 10 ml + 8g sucrose

Pasteurized for soln @ 60°C

Freeze immediately with carry out SDS PAGE ASSAYS

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## CAE Analyses on AHF

- |   |                   |   |       |       |
|---|-------------------|---|-------|-------|
| 1 | Albumin           |   |       |       |
| 2 | Albumin           |   |       |       |
| 3 | M 3094            | } AHF Lt that<br>was pasteurized 2021-48A | Fibr. | 8966  |
| 4 | M 3094            |   | 32.7% | 67.2% |
| 5 | C-1051            | } AHF sterile<br>serum 2021-45            | 33.7% | 62.2% |
| 6 | C-1051            |   | 63.3% | 36.6% |
| 7 | } Paul Hys sample |   | 61.2% | 38.7% |
| 8 |                   |   |       |       |

## Effect of Fibrinogen content

1 vial of K4724 Paragen (Exp 15 Oct 1974) was reconstituted and  
saturated with sucrose (5 gms/10 ml). Gelation occurred  
within 30 mins. @ 60°C.

## Paragen additives

- 1 gm Fibrinogen
- 0.92 gm Sodium citrate
- 2.5 gm dextrose
- 60 mg glycine
- pH adjusted with HCl/NaOH
- No preservatives

reconstituted in 50 ml WFI

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## Effect of Fibrinogen content on gelation time for AHF

2 vials of Kaste M3094 Reconstituted in 20 ml WFI  
1 vial of Paragon K4724 Reconstituted in 50 ml

Sample #	Ant. of AHF (2.0% increase)	Ant. of Fibrinogen (2.0% increase)	Ant. removed for CPE	Ant. heated 60°C	Gelation Time
1	6 ml	0	1 ml	4 ml	
2	5 ml	1 ml	1 ml	4 ml	3.7 hrs
3	4 ml	2 ml	1 ml	4 ml	1.63 hrs
4	2 ml	4 ml	1 ml	4 ml	48 min
5	1 ml	5 ml	1 ml	4 ml	17 min
6	0 ml	6 ml	1 ml	4 ml	17 min

## CRE Analysis

Sample #	% $\beta$ Globulin	% Fibrinogen
1	66.5	32.5
2	43.5	56.5
3	37.7	62.3
4	19.9	80.1
5	14.1	85.9
6	6.6	93.4

2.0% Albumin  
Albumin standard

Albumin

100%  
100%

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## Pasteurization of Konyne

Konyne M 5542 (Exp. 19 July 78) reconstituted in 20 ml.  
 Saved 2 ml for CAE, protein. Saved 8 ml for assay (2021-52A).

Pasteurized from 10:20 am to 8:30 pm @ 60°C.

Volume = 4 ml.

Frozen 2 ml for assays (2021-52B)

Dialyzed 12 ml against 15L of f.c. buffer:

2. 0.0925 M NaCl (5.41 g/L)

0.0525 M sodium citrate (15.44 g/L) pH 7.3.

from 8:30 pm 1/2 to 8 am 1/9

Dialyzed volume 43 ml.

(2021-52C)

2021-52A

$A_{280} = 1.116 \times 25 = 27.9 A_{280} \text{ units}$

2021-52C

$A_{280} = 0.2 \times 25 = 5 A_{280} \text{ units}$

2021-52B

Raw RBD Coag data

1X unit

14.3 units

34 units/l

0

0

Inhibitory

0.005

NAPIT ↑

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RJF

MIL 028903

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## Pasteurization of AHF

4 vials of Koate M 30% reconstituted in 40 ml.  
 Removed 2.5 x 2 ml, 1 x 1 ml as control samples.  
 Volume remaining 34 ml. Added 28 g. sucrose.  
 Volume after sucrose added: 48 ml  
 Control volume 18 ml left @ 5°C for entire period  
 Balance 30 ml pasteurized begin 9 AM + 10 min  
 25 ml dialyzed  
 vs. 3M glycine  
 15M NaCl  
 0.1M Sodium Citrate  
 1% dextrose

also dialyzed (in same tank) 11 ml control  
 volume after dialysis  
 Control 43 ml  
 pasteurized 58 ml  
 feed material

A <sub>580</sub> *	A <sub>580</sub> (1:25)
.024	.145 x 25 = 3.625
.115	.098 x 25 = 2.45
	.085 x 25 = 2.125

Note: blood spots in buffer. On outside of bag: it counted from  
 Control with. (would not go in .454 NaCl)

Submitted to Coagulation Lab for TIME, VIII C: WF

- 2021-52A Koate reconst. frozen no treatment
- 2021-52B Koate + sucrose at 5°C 10 hr.
- 2021-53C Koate + sucrose at 61°C 10 hr.
- 2021-53D B dialyzed 13 hr
- 2021-52E C dialyzed 13 hr

\* A<sub>580</sub> taken after freezing + thawing

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RTG

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~~28 ml dialyzed~~  
 VS: .3M glycine  
 .15M NaCl  
 .01M Sodium Citrate

and 7:10.2m

Results from AHF Pasteurization Expt.

Raw data from Coag. R & D Lab:

2021-53A	36.6	VI C units/ml	2
-53B	17.8		3
-53C	12.5		3
-53D	5.1		3
-53E	4.6		3

Specific Activity VIIC

2021-53A	2.152
-53B	1.478
-53C	1.039
-53D	1.406
-53E	1.877

Reassay

2.1.355

2.1.355

62.5%

Feed 2.152 VI C / 100% 100%

lucrose

60°C, 10 hours

5°C, 10 hours

1.938 VI C / 100% 79.2%

1.478 VI C / 100% 68.6%

DIALYSIS - 13 hrs, 5°C  
 against fc buffer

1.877 VI C / 100% 87.2%

1.4068 units / 100% 65.4%

On reassay:  
 2021-53A 22.2 VI C  
 2021-53B 2 VI C

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## Pasteurization of Kongre

### ANF Buffer

0.3M Chlorine

0.15M NaCl

0.01M Sod. Citrate

1% Dextrose

### Kongre Buffer

0.04M Sod. Citrate

0.08M NaCl

## Discussion with Bob Lurie

Pasteurization @ 60°C, 10 hrs does not necessarily destroy antigenicity - Use SV-40 (stable @ 56°C for 1-2 hrs)

5 x 20 ml (approx. vol.) of Kongre K7331 (at 27, 73) were mixed together. 5 x 2 ml aliquots of this feed material stored at -70°C. Balance of 86 ml of vol. not added to 110% sucrose. Dissolved completely and pasteurized for 10 hrs @ 60°C. Total volume after sucrose add: 150 ml.

Diafiltered 100 ml against f.c. buffer. Diafiltered 50 ml against f.c. buffer for 4 hrs.

2021-56A - feed material K7331, Kongre.

2021-56P - post diafiltered material (vol. 200 ml)

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Dec 17 1955 for Karpis pasteurization study  
CAE of various Karpis lots

	Batch	Albumin	Bilirubin	Fibrinogen
M 2835	(1)		66.1	33.9
	(2)		63.8	36.3
	Mean		64.9	35.1
PK 2552	(3)	11.7		
	(4)	<del>11.8</del>	36.4	51.9
		11.8	40.7	47.9
	Mean	11.8	38.6	49.6
NC 8004	(5)		41.6	58.4
	(6)		41.2	58.8
	Mean		41.4	58.6
A 3143	(7)		56.3	43.1
	(8)		61.9	38.0
	Mean		59.4	40.7

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## Hepatitis Antigen Study

10 ml of desalted PR 2712 was antiserated with sucrose (2g) and spiked with surface Ag. (Micro R & D). The sample was pasteurized 60°C, one and given to Micro R & D for assay.

2021-57 feed

2021-57, 60°C, 1 hour

Hepatitis B virus

1:16

negative

## CEP Test

A<sub>6</sub> A<sub>9</sub>

1% Agar

controls  
2 deletions

+	0.1%
+	0.1%
+	0.1%
+	0.1%
+	0.1%
+	0.1%
+	0.1%
+	0.1%
+	0.1%
+	0.1%

micro R & D spiked  
MISG with purified  
HBs Ag

1:2

1:4

1:8

1:16

1:32 etc.

## Fibrinogen Assay

C-1051 sup:

A<sub>50</sub> 1.30 - 0.633

≡ A<sub>50</sub> of 31.65 units

Saline 57 ml

50 ml of C-1051. Thawed.

sampled 25 ml + 2 ml 2021-57.

This 57 ml of C-1051 at A<sub>50</sub> of 31.65 was diluted to an A<sub>50</sub> (calculated) of 10 by addition of 145 ml of f.c. buffer.

f.c. buffer 0.2M glycine

0.15M NaCl

0.01M 2nd buffer

1% bovine

pH 9.3

ABSTRACT

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Am't of glycine required (1.6M) = 120 gm/L

Am't of solution = 145 - 67 = 212 ml = 0.212L

Am't of glycine 25.4 gm

This was added to the soln in the 5°C. Reaction 45 mins.

Removed 15 ml (2021-578)

Concentrate to ~100 ml. Decaffinated to ~1 vol. replacement  
and freeze 2021-57C aged for buffer  
P (UF problems)

MISG - C<sup>4</sup> Expt

2021-58

Used BPR

1-24-77

Defatted Albumin polymerization (1948-92)

Results

1. Defat Albumin + 8 gm sucrose - (no heat)

Inner  
Monomer  
27.58%

2. 10 ml defat Alb + 2 gm sucrose (heat)

17.8%

3. 10 ml defat Alb + 4 gm sucrose (heat)

27.5%

4. 10 ml defat Alb + 6 gm sucrose (heat)

34.4%

5. 10 ml defat Alb + 8 gm sucrose (heat)

37.18%

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## Hepatitis Antigen study on Korte

### Design of experiment

Korte used: M2835 (Nov 75) 19.5 units/ml, 2.0079 g/ml (labeled)

### Contents

### Sample #

F.VIII in sucrose (feed)

2021-59A

F.VIII in sucrose (pasteurized)

2021-59B

F.VIII in sucrose pasteurized  
followed by post addition of  
HBsAg at same concentration

2021-59C

Sucrose in Antigen (feed)

2021-59D

Pasteurized sucrose in  
Antigen

2021-59E

### Results

F.VIII spiked with HBsAg shows a full drop in titer.  
While anti. sucrose (no Korte) spiked with HBsAg shows  
a constant titer. HBsAg apparently binds nonspecifically  
to protein at elevated temperatures not demonstrated  
at +50°C.

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## Experimental Design for AHF Pasteurization experiment

2000 ml pH adjusted past salted AHF : Identification # 8M 7364

### Steps

- ① Remove 6x4 ml Label 2021-61A - Measure A280, 50
- ② Add sodium citrate 2.94 g/L
- ③ Add solid Glycine 120 g/liter initial T = 10°C
- ④ React 1 hour
- ⑤ C.F. @ 50

Supernatant 4x4 ml Label 2021-61B - Measure A280, 50  
 Paste weight: Label 2021-61C

- ⑥ Difilter against 0.0M sodium citrate, 0.1M glycine for 2 replacements and 0.1M NaCl

Retention	Initial Level	glycine	sodium citrate
0		1.6M	0.02M
1 replacement		0.58M + .067	0.072 + .006 = 0.013
		= 0.647M	
2 replacements		0.216 + 0.086	0.0027 + 0.006
		= 0.31M	= 0.011M

Concentrate to A280 = 31  
 save 60 ml

15x4 ml

2021-61D

I affirm that the above is a true record of work done by me

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Date 1-29-79

INT 872

MIL 028912

# CUTTER Laboratories, Inc.

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Continued from Page

2am 1/30 Put salt AHF (6M 7364), pH 6.94  
 Tol 1.75L Rouch 6x4ml 2021-61A  $A_{250} = 0.522$   
 Balance 1.925L, -9°C Total feed base 8.05  
 Citrate required  $2.94 \times 1.925 = 5.66g$  - Mixed for 5min.  
 Glycine (1.6M)  $= 120 \times 1.925 = 231g$  1 hour Temp dropped to  
 Glycine addition completed 2:30am. 4°C. Ran in + 5°C Room  
 Centrifuged @ 5000 rpm for 30 min. using 1L bottles @ 5°C  
 Collected 2026ml of supernate. Filled 6x4ml 2021-61B  
 Diffused 2000ml Amicon 1ft hollow fiber cartridge HIP10 against  
 0.1M glycine  
 0.01M succinate  
 0.15M NaCl  
 Diffusion start ~4:30am FR ~750ml/hr. (Initial)  
 Assoc of succinate after replacement  $A_{250} = 0.24 = 6 \text{ units}$   
 Diffusion stopped 7:10am Volume diffused = 3L  
 UF started 7:10am  
 UF stopped 8:05am Volume concentrated to 540ml  
 Final  $A_{250} = 0.80 = 20 \text{ units}$   
 10ml - assay ~15x4ml 2021-61B  
 saved 6x50ml  
 Vial 200ml + 195g sucrose pasteurized 2.0am to 6:45pm  
 SAMPLE AFTER 7AM NOT DIAL = 2021-625 (pH 6.79)  
 Dialyzed vs 60L BUFFER (.3M GLYCINE, .15M NaCl, .01M Na Citrate)

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Date

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# CUTTER Laboratories, Inc.

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Page No.

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Dialyzed volume = 1060 ml

1000 ml Concentration start 8:00 am.  
ultrafiltered to 178 ml.  
Concentration completed 7:10 pm.

60 ml  
final sample  
(2021-62F)

Assay 10.168

The ultrafiltered samples are designated 2021-62G

Filled 15 x 4 ml + 10 x 10 ml + 1 x 25 ml

## CAE Assays

2021-61A Post salt soln.

Alb	B	Fib
39.7	23.2	37
40.4	7.8	41.7
37.7	23.2	34.1
Mean	21.4	39.3

2021-61D Post Glycine / d.f. / step

45.4	22.2	32.2
48	10.3	31.7
47.5	10.9	31.6
Mean	11.1	31.8

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Assay results on 2021-61

1 sample 4/5/79

Step		Volume	A <sub>280</sub>	Y <sub>III</sub> C y/ml	Total y <sub>III</sub> C	y <sub>III</sub> C / ml	Total
Post salt step: Alpine super UF	2021-61A	1.825 L	5.03	3.6	6930	3	5775
	2021-61B	2.026 L	6.525	3.3	6686	3.2	6483
	2021-61D	547 ml (corrected) (270 + 2.026) L	22.35	8.8	4813	13.8	7549
Dialysate	2021-61D	200 ml	20.35	8.8	1760	13.8	2760
	2021-61F	1060 ml	5	1.1	1166	1.6	1676
Post concentrated		189 ml (corrected) (178 + 1.16)	23.77	4.5	851	4.5	851
						7.8	1474

Corrected analyses using all the 2021-61 material

2021-61D, corrected : 547 ml  
2021-61F, 2899 ml  
2021-61G 516.9 ml

Total y<sub>III</sub> C      Total  
4813      7549  
3189      4638  
2326      2326  
4031.2

Recovery across pasteurization step

Y<sub>III</sub> C :  $\frac{3189}{4813} \times 100 = 66.25\%$   
V<sub>III</sub> WF :  $\frac{2638}{739} \times 100 = 61.44\%$

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4/5/79

JUL 2 1979

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# CUTTER Laboratories, Inc.

91

Page No.

Research Record Book No. 2621

Continued from Page

16-1-156  
 Selective destruction of Fibrinogen in AHF by heat  
 2 vials of PR 2552 reconstituted. Vol 21 ml. Added 1.7g sucrose. Vol 30 ml. Centrifuged 5ml for assay. 8.45 am 1:30 pm  
 supernatant vol 17 ml  
 1 20 ml vol of M 3143 reconstituted in 20 ml fibrinogen sol (from lyophilized Fibrinogen bottle K 4061). Added 1.7g sucrose. Centrifuged 5ml sample. Heated 60°C prior to assay. Cellulose by 1 pm. Fuged at 15,000 rpm, 30 min. Supernatant vol 14 ml. Sampled.

	Albumin	$\beta$ globulin	Fibrinogen
PR 2552 (feed)	0.45%	44.09	55.45
(Treated)	19.05	58.33	22.61
3143 feed		25.75	74.24
Treated		63.74	36.2

Sample submitted for  $\overline{M}_w$

PR 2552 feed 2021-91A  
 PR 2552 treated 2021-91B

Continuation of results on C vs P MISL from Pg 84

SDS PAGE

	22mer	H <sub>2</sub> L <sub>2</sub>	H <sub>2</sub> L	H <sub>2</sub>	H <sub>2</sub> L	H	L
2163-31P	1.4	3.3	3.4	3	7.4	53.7	273
2163-31C	0	3.2	4.6	5	8.1	52.5	266

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Date

5/10/74

JUL 2 1974

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PLAINTIFF'S  
EXHIBIT

974

PLAINTIFF'S  
EXHIBIT

10

Fernandes  
1-25-97

utter Laboratories, Inc  
MEMORANDUM

TO: J. Gible  
FROM: Peter Fernandez  
DATE WRITTEN: 10-11-79  
SUBJECT: Protein Stabilization During Pasteurization

COPIES TO:

B. Bradley  
M. Moxen  
V. Cabasso  
J. Lundblad

RECEIVED

JAN 12 1979

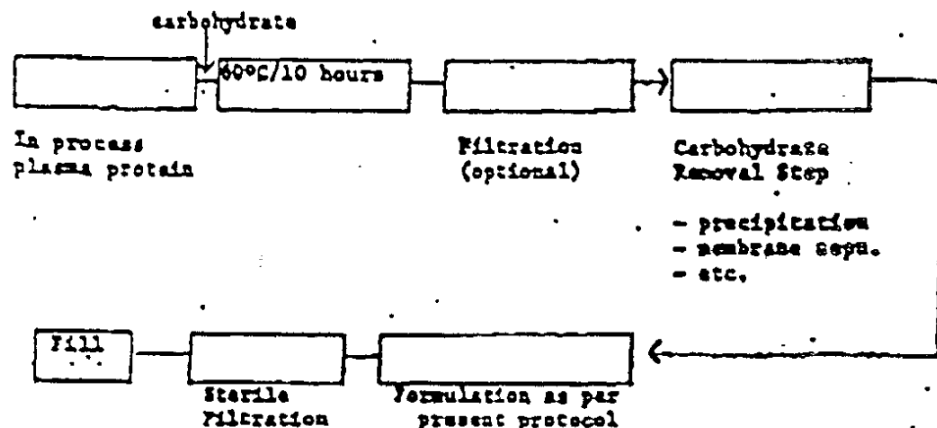
PATENT, TRADEMARK  
& LICENSING DEPT.

This memo is being sent to inform you on progress we have made in reference to our patent disclosure - "Pasteurization of Fibrinogen, Factor VIII, Factor IX, Gamma Globulin, Plasminogen, CIG and other plasma proteins in the presence of carbohydrates" - Peter Fernandez and John Lundblad, 10/25/78, amended 12/14/78.

As was stated in the disclosure we have been directing our attention to inactivating the hepatitis virus by heat treatment at 60°C for 10 hours. With our present processes, none of the above products (with the exception of plasminogen) can be pasteurized because they are very heat labile. Even though the source plasma is tested for hepatitis surface antigen, the test is not sensitive enough and hence all non-pasteurized material (with the exception of Cohn Method 669 processed gamma globulin) are always suspect in terms of hepatitis contamination.

Our work in this area has shown that many of these materials can be bulk pasteurized in the presence of certain carbohydrates - the effectiveness of the step being dependent not only on the type of sugar but more importantly on its concentration. The carbohydrate is then removed after pasteurization by standard methods (diafiltration, dialysis, etc.) so that the final container material is identical to that of the present formulation for the product.

A schematic of our proposed process is shown below:



PLAINTIFF'S  
EXHIBIT

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EX. 625 09691

PLAINTIFF'S  
EXHIBIT

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All processing downstream of the pasteurization would have to be in a hepatitis free area.

The following carbohydrates have been investigated:

Dextrose 10% to saturation  
Maltose 10%  
Sucrose 10% to saturation.

Emphasis was placed on sucrose based on Seeger's comment in "Purified Prothrombin & Thrombin: Stabilization of Aqueous Solutions" Arch Biochem 3, 363 (1944) that saturated sucrose could protect thrombin when heated at 50 C for 48 hours with only a 3% loss in activity. His work was not concerned with the preparation of clinical material.

Our results to date are shown below:

1. MISC (ISC) - Pasteurized 60°C, 10 hours in saturated sucrose

Test	Observed	Reference Value	
AC activity	8.4mg/CH50	Succ. NLT 3mg/CH50	
GFC-gel permeation chromatography, I		PR Ing	Mean Values
		in 0.1M Gly, 0.45% NaCl	in 30% Maltose, 0.1 M Glycine
> dimer	6.7	7.92	1.54
dimer	7.4	15.10	8.60
Monomer	85.9	75.10	88.21
< Monomer	0.7	1.85	1.50
SDS-PAGE			
H <sub>2</sub> L <sub>1</sub>	7.4		
H <sub>2</sub> L	7.6		
H <sub>2</sub>	9.9		
KL	11.6		
K	37.5		
L	26.1		
Antibody Potency		PR Lot	Mean Values
Measles	0.3		0.3
Polio	0.8		0.3
Diphtheria	>1<2		>1<2

These results (although not a complete characterization) do indicate that a pasteurized MISC (ISC) can be made which is indistinguishable from the current product as observed by our release criteria.

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Factor VIII

Our work in this area is very much in its preliminary stages. We have been working exclusively with lyophilized material from old production lots because of its easy accessibility. Initially we saw significant variation in the ability of Koate® to survive pasteurization even in the presence of sucrose. This was tracked down to the level of fibrinogen in the product. The table below shows the effect of fibrinogen on gelation time at 60°C.

<u>Sample #</u>	<u>IgG Globulin</u>	<u>I Fibrinogen</u>	<u>Gelation Time, hours</u>
1	6.6	93.4	0.28
2	14.1	83.9	0.28
3	19.9	80.1	0.80
4	37.7	62.3	1.63
5	43.5	56.5	3.7
6	66.5	33.5	> 10

Material containing less than 33% fibrinogen is not available.

Our preliminary run (2021-48) with Koate® (03094) resulted in a recovery of 45% VIII:C activity with a specific activity of 0.7 VIII:C units/A<sub>280</sub>. VIII:K activity assays are in progress. It is felt that with optimal processing and handling techniques (example: diafiltration instead of dialysis as was used here) the yield can be improved. With the impending availability of Maxwell processed Factor VIII with very low fibrinogen, the prospect of a successful pasteurization process appears bright.

Factor IX, Defatted Albumin

With these products, we have shown that pasteurization is possible while maintaining visual clarity. Activity assays are in progress.

We will continue to keep you informed as more data become available. ✓

The utility of pasteurizing gamma globulin may at first seem unnecessary since gamma globulin made by Cohn's method 6B has an excellent record as being non-hepatitic. This, of course, has led to its widespread clinical use. However, this fact has also hindered the development of newer methods for manufacturing albumin since gamma globulin prepared by a non-Cohn process is always suspect in terms of its ability to transmit hepatitis. A method for pasteurizing gamma globulin would be useful in this connection.

In our initial disclosure we included the pasteurization of Fibrinogen, but our data on Koate® pasteurization leads us to believe that we as yet cannot pasteurize this protein. ✓

FF:jd

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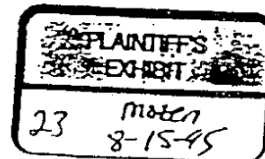
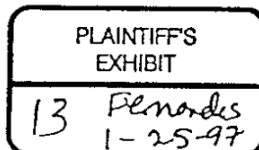
Application for United States Patent

Inventors: PETER M. FERNANDES  
JOHN L. LUNDBLAD

Invention: PASTEURIZED THERAPEUTICALLY  
ACTIVE PROTEIN COMPOSITIONS

Abstract of the Disclosure

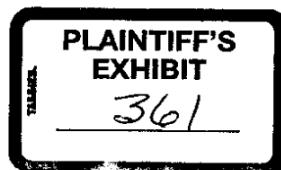
Compositions containing thermally sensitive, therapeutically active proteins are pasteurized without substantial loss of therapeutic activity by mixing the protein composition with a pasteurization-stabilizing amount of a sugar or reduced sugar and of an amino acid prior to pasteurization. Pasteurized compositions containing therapeutically active proteins, which have heretofore been unattainable, can be prepared by the method of the invention.



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Cross-references to Related Applications

This is a continuation-in-part of U.S. Patent Application Serial No. 202,508, filed October 31, 1980, which is a continuation-in-part of U.S. Patent Application Serial No. 127,351, filed March 5, 1980, now abandoned

Specification

Background of the Invention

1. Field of the Invention: This invention relates to and has among its objects novel compositions for therapeutic use and methods of making them. It is a particular object of this invention to provide pasteurized compositions containing therapeutically active proteins. Further objects of the invention will be evident from the following description wherein parts and percentages are by weight, unless otherwise specified.

2. Description of the Prior Art: Many useful blood fractions and blood proteins are obtained from human blood plasma by fractionation according to known techniques such as, for example, the alcohol fractionation method of Cohn described in U.S. Patent No. 2,390,074 (1945) and the Journal of the American Chemical Society, Vol. 68, page 459 (1946) and the Rivanol® ammonium sulfate method. The aforementioned methods as well as other variations and techniques are summarized in "The Plasma Proteins", second edition, Volume III, pages 548 - 550, Academic Press, New York, New York (1977). These blood fractions contain biologically active proteins that possess certain therapeutic qualities. For instance, Factor VIII or antihemophilic factor is useful against hemophilia; plasminogen is a precursor of plasmin for treatment of acute thromboembolic disorders; immune serum globulin (IgG) is employed in the treatment of congenital gamma globulin deficiency, measles, poliomyelitis and hepatitis A and B;

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fibronectin has been identified as active in treatment of burns, shock, cancer, etc.; antithrombin III is a coagulation inhibitor, cryoprecipitate itself may be used directly for classic hemophilia; Plasma Protein Fraction (human) and albumin are useful in treatment of shock due to burns, crushing injuries, abdominal emergencies, and any other cause producing a predominant loss of plasma fluids and not red cells; immune globulin, intravenous (modified immune serum globulin) is a substitute for immune serum globulin administerable in larger quantities; Factor VIII inhibitor bypassing active (FEIBA) substance described in U.S. Patent 4,160,025 as a blood-coagulation-promoting preparation for Factor VIII inhibitor patients; alpha-1-antitrypsin can be employed in the treatment of emphysema; plasma growth hormone corrects pituitary growth deficiency, somatomedin is useful in correcting growth deficiencies, other immune serum globulins, e.g., IgA, IgD, IgE, and IgM, may be employed to treat various immune protein deficiencies; prealbumin (U.S. Patent 4,046,877) is employed to increase immunologic competence; plasminogen-streptokinase complex (U.S. Patent 4,178,368) can be administered to patients for treatment of thromboembolisms; ceruloplasmin, transferrin, haptoglobin, and prekallikrein have reagent and other uses.

One problem confronting users of plasma, plasma fractions, and compositions containing individual blood proteins is the thermal instability of the therapeutically active proteins contained therein. In many cases, substantial, and sometimes complete, losses of activity are observed if these proteins are heated above physiological temperatures, i.e., above about 40 - 45° C. Consequently, these items require special care during preparation and storage to minimize such deactivation.

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The thermal instability of the aforementioned proteins renders them unpasteurizable. Therapeutically active

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proteins isolated from plasma may contain viruses, e.g., hepatitis virus, present in the source material for the protein fraction, namely, blood from a donor. A risk of contracting hepatitis exists, therefore, for those receiving unpasteurized fractions from blood plasma fractionation because the presence of the virus cannot be detected with certainty by any known procedure. In a large number of situations, this risk is outweighed by the detriment to a patient in not receiving the therapeutic plasma fraction as determined by the physician.

Some therapeutically active proteins derived from plasma have been pasteurized successfully. For example, it is well known that albumin can be pasteurized by heating at 60° C or 64° C for 10 hours (Gellis et al, J. Clin. Invest., Vol. 27, pages 239 - 244 [1948]) in the presence of certain stabilizers such as acetyl-tryptophan and sodium caprylate. Individuals receiving this pasteurized material did not contract hepatitis, thus indicating the inactivation of hepatitis virus while retaining the activity of albumin under the afore-described heating conditions. Plasma Protein Fraction (human) is also stabilized during pasteurization by the above method.

A process for pasteurizing plasminogen is disclosed by Baumgarten et al in U.S. Patent 3,227,626. An aqueous preparation containing 0.25 - 20 milligrams per milliliter (mg/ml) of plasminogen and further containing 0.1 - 0.5 molar lysine with a pH of 5.3 - 7.5 was heated at 60° C for 10 hours. As the patentee states, hepatitis virus was destroyed and the danger of transmitting hepatitis was removed with retention of plasminogen activity. Attempts to pasteurize plasminogen under the above conditions in the absence of lysine resulted in complete destruction of plasminogen cannot be stabilized with N-acetyl-tryptophan and sodium caprylate during pasteurization, nor can albumin

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and Plasma Protein Fraction (human) be pasteurized in the presence of lysine.

Singher has described a process for treating plasminogen to produce a material that is not contaminated with hepatitis virus (U.S. Patent 2,897,123). In the patented pasteurization technique aqueous solutions of plasminogen are heated at about 60° C for about 10 hours. The activity of plasminogen is retained if the solutions have a pH in the range not less than 3 nor greater than 6.5 and an ionic strength not greater than 0.3.

Another method for removing hepatitis virus from a biological material is described in U.S. Patent 4,168,300. The material to be treated is contacted with a preparation, which may be agarose gel or beaded polyacrylamide plastic coupled with a variety of hydrophobic ligands. Plasma and albumin were subjected to the above purification technique to remove hepatitis virus.

Aqueous solutions of the enzyme thrombin have been stabilized (Seegers, Arch. Biochem., 1944, Vol. 3, pages 363 - 367) during heating at 50° C in the presence of saturation amounts of certain glycosides. The stabilized solutions were heated at the above temperature for a period of 48 hours or more with minimal loss of activity. On the other hand, Seegers also discloses that glycosides and polyols have only minimal effectiveness in stabilizing the enzyme prothrombin. The reversible denaturation of lysozyme and ribonuclease was studied by Gerlsma et al., Int. J. Peptide Protein Res., Vol. 4, pages 377 - 383 (1972). The authors found that certain polyhydric alcohols increased somewhat the temperatures at which these enzymes were denatured. Finally, Simpson et al., in J. Am. Chem. Soc., Vol. 75, No. 21, pages 5139 - 5152 (1953) and Donovan in J. Sci. Fd. Agric., Vol. 28, pages 571 - 578 (1977) noted that the denaturation temperature of ovalbumin (an

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egg white protein) was raised slightly in the presence of sucrose in aqueous solutions of the protein. However, Donovan points out that the temperatures of denaturation of ovalbumin and S-ovalbumin are 84.5° C and 92.5° C, respectively. Furthermore, ovalbumin and S-ovalbumin, as well as the aforementioned enzymes, have no therapeutic activity in treating disorders in humans, whereas blood plasma proteins are therapeutically active. In fact, as mentioned below, proteolytic enzymes deactivate blood plasma proteins.

Singher, in the aforementioned U.S. Patent, lists some methods of destroying hepatitis virus. The least effective of these methods involves the use of either nitrogen mustard or beta-propiolactone. High energy irradiation in appropriate dosage is effective but destroys biological activity when applied to human blood products. Heat is recognized also as effective against hepatitis virus, the preferred treatment being heating the material at 60° C for 10 hours. Higher temperatures above 70° C for shorter intervals or lower temperatures for longer intervals have also been tried with successful results. However, it is important to note that higher temperatures are undesirable because of the potential for denaturation of the proteins. Furthermore, lower temperatures for long intervals are to be avoided because various proteolytic enzymes are activated under these conditions, and these activated enzymes cause protein degradation. Also, the use of temperatures lower than 60° C for pasteurization has not been shown to consistently yield a material that does not contain the infective virus.

As mentioned above, the recognition that heating at 60° C and 64° C for 10 hours successfully destroys the hepatitis virus in albumin was made by Gellis et al, supra. Gellis et al proved experimentally that albumin heated under the above conditions did not transmit hepatitis even if

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hepatitis virus was present prior to pasteurization. However, the author noted that hepatitis virus survived heating at 56° C for one hour, a temperature usually employed for the inactivation of viruses. Thus, although heating at temperatures of about 56° C for one hour will deactivate most viruses, hepatitis virus is not inactivated; and materials containing hepatitis virus, which are heated at 56° C for one hour, cause infection of hepatitis in individuals receiving such materials.

#### Summary of the Invention

The invention described herein provides means for obviating the above-outlined problems. In the method of the invention certain compositions containing thermally sensitive, therapeutically active proteins are rendered heat stable during pasteurization or heating at a temperature of about 60 - 75° C by mixing with heat-stabilizing, or pasteurization-stabilizing, amounts of a sugar or reduced sugar and an amino acid. Pasteurized compositions containing therapeutically active proteins heretofore unobtainable are available as a result of the process of our invention by heating a mixture of unpasteurized protein composition, a sugar or reduced sugar and an amino acid suspended or solubilized usually in an aqueous medium at a temperature and for a time sufficient to pasteurize the protein composition. Following pasteurization or heat treatment, the sugar or reduced sugar and amino acid are removed totally or in part, as desired, from the protein composition by conventional techniques, and the pasteurized protein composition is processed according to conventional procedures for its ultimate therapeutic use.

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The primary advantage of the invention is the availability of thermally stable and pasteurized therapeutically active protein compositions, which heretofore have been unknown

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In the method of the invention, the protein composition to be pasteurized is suspended or dissolved in an aqueous medium with an amount of sugar or reduced sugar and of amino acid sufficient to stabilize the protein composition during subsequent pasteurization. The concentration of ~~polysaccharide~~ sugar or reduced sugar and of amino acid necessary to stabilize a protein composition in accordance with this invention depends on the type and concentration of therapeutically active protein in the protein composition, on the type of sugar or reduced sugar used, and on the amino acid used. The therapeutically active protein is considered to be stabilized if it retains a substantial portion, i.e., at least 40%, of its therapeutic activity during pasteurization. It is preferred that 70% or more of the therapeutic activity of the protein composition be retained during pasteurization. Consequently, the amount of sugar or reduced sugar and of amino acid to be added should be such as to retain the above-recited amount of therapeutic activity.

Typical examples of sugars that may be employed in our method are mono-, di-, and trisaccharides such as arabinose, glucose, galactose, fructose, ribose, mannose, rhamnose, sucrose, maltose, raffinose, <sup>^</sup>melezitose, and so forth. Exemplary of reduced sugars included within the purview of the invention are erythritol, ribitol, sylitol, sorbitol, mannitol, etc. <sup>\* below</sup> Generally, the amount of sugar or reduced sugar used in combination with amino acid to stabilize the protein composition during subsequent pasteurization should be at least about 0.8 g/ml (54% w/v, 45% w/w) based on total aqueous solution or suspension of the protein composition, sugar or reduced sugar and amino acid. A useful range of amounts of sugar or reduced sugar in the method of the invention is about 0.8 g/ml to about 1.5 g/ml. Although the mentioned useful range is preferred, still greater amounts of sugar or reduced sugar may be used to achieve the advantages of the method of the

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\* Preferably, the sugar or reduced sugar should be water miscible and physiologically compatible with the protein and have a low molecular weight, i.e. a molecular weight less than about 5000.

DEP 063013

invention. Sucrose is preferred as the sugar or reduced sugar compound.

As the amino acid one may employ lysine, arginine, leucine, iso-leucine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, aspartic acid, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, and the like and mixtures thereof. Substances producing the aforesaid amino acids such as an amino acid salt and the like also may be used. It should be understood that amino acids in the absence of a <sup>sugar or reduced sugar</sup> ~~polymer~~ are not effective pasteurization-stabilizing agents for those protein compositions that have been unpasteurizable prior to this invention. Generally, the amount of amino acid used in combination with sugar or reduced sugar during subsequent pasteurization may be in the range of about 0.04 M to about 0.8 M, based on aqueous solution of protein composition, sugar or reduced sugar and amino acids of at least one amino acid. At least one of arginine, lysine and glycine is preferred as the amino acid.

Generally, the amount of protein composition in the aqueous mixture of protein composition with sugar or reduced sugar and amino acid will be in the range of about 1 mg/ml to about 15 mg/ml. However, the amount of protein composition is not believed to be critical.

After the protein composition has been mixed with the sugar or reduced sugar and amino acid, the mixture is heated at a temperature and for a time sufficient to pasteurize it. Thus, the mixture is pasteurized upon heating it under conditions known to inactivate hepatitis virus. Effective pasteurization to inactivate hepatitis virus and to substantially reduce the risk of hepatitis infection is obtained by heating an unpasteurized protein composition at a temperature of about 60 - 75° C, preferably about 60 -

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DEP 063014

70° C for a period of about 10 hours, usually about 62° - 65° C for about 10 hours.

The pasteurization is carried out under pH conditions which approximate physiological conditions. Thus, the pH of the mixture usually should be within the range of about 5.5 - 8.0, preferably about 6.0 - 7.5. The general, physiological conditions are desirable, where possible, during pasteurization to insure the least disturbance to the therapeutically active protein composition.

The amounts of a particular sugar or reduced sugar and amino required to stabilize a specific protein composition during pasteurization and the conditions necessary to pasteurize the composition can be determined readily by one skilled in the art using pilot trials in accordance with the teaching contained herein.

Following pasteurization the mixture of sugar or reduced sugar, amino acid and protein composition may be treated to remove all or part of the sugar or reduced sugar and amino acid. Conventional techniques can be employed to achieve this end. For example, the mixture can be dialyzed or diafiltered using an appropriate semi-permeable membrane. Other means of removing the sugar or reduced sugar and amino acid will be suggested to those skilled in the art.

The pasteurized mixture may be treated to remove water therefrom by procedures well known in the art. For instance, the mixture can be freeze-dried or ultrafiltered and then freeze-dried. Furthermore, the mixture can be sterile-filtered by conventional methods prior to water removal.

The pasteurized protein compositions of the invention can be formulated into pharmaceutical preparations for therapeutic use. To prepare it for intravenous

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DEP 063015

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administration the protein composition is dissolved usually in water containing physiological substances such as sodium chloride, glycine, and the like and having a buffered pH compatible with physiological conditions. Generally, guidelines for intravenously administered protein compositions are established by governmental regulation.

*included within the scope of the invention are these proteins*  
Thermally sensitive, therapeutically active proteins generally administered to patients for preventative and/or curative purposes, which lose some therapeutic activity when heated above about 40 - 45° C and which are capable of being stabilized during pasteurization or heating at a temperature of about 60 - 75° C in the presence of a polyol (i.e. a polyhydroxyl compound, for example, polyhydric alcohols and carbohydrates such as sugars). Examples of therapeutically active proteins that may be pasteurized in accordance with the present invention, by way of illustration and not limitation, are those proteins derived from venous blood plasma or placental plasma and include blood plasma, partially fractionated blood plasma proteins. Thus, for example, protein compositions pasteurizable by the method of the invention may include as the therapeutically active protein plasminogen, albumin, anti-hemophilic factor (Factor VIII), Plasma Protein Fraction (human), fibronectin (cold insoluble globulins), an immune serum globulin such as IgG, IgA, IgD, IgE, and IgM, high molecular weight kininogen (90,000 - 106,000), an immune globulin, intravenous (modified, either chemically or enzymatically or by fractional separation, immune serum globulin), FEIBA, antithrombin III, alpha-1-antitrypsin, plasma proteins (molecular weight 1000 - 30,000) having growth activity such as plasma growth hormone, somatomedin, prealbumin, plasminogen-streptokinase complex, ceruloplasmin, transferrin, haptoglobin, and prekallikrein, etc., and mixtures thereof. In addition, pasteurized compositions containing "defatted" albumin and "defatted" Plasma

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Protein Fraction (human), i.e., PPF (human), are available through the invention. The term "defatted" means that the albumin and PPF (human) contain no more fatty acid material than that present in the native state prior to pasteurization. The pasteurized defatted compositions can be administered to patients who cannot tolerate infusion of high fatty acid material such as that obtained using standard pasteurization stabilizing agents, namely, sodium caprylate and sodium acetyl-tryptophanate.

It is noteworthy that antihemophilic factor B (Factor IX) and prekallikrein activator cannot be pasteurized in the presence of a polyol in accordance with the above method. Indeed, these proteins lose substantially all their therapeutic activity under conditions under which the aforementioned protein compositions retain a substantial portion of their activity.

In particular, the method of this invention is directed to pasteurizing a composition comprising a thermally sensitive, therapeutically active protein selected from the group consisting of antihemophilic factor (Factor VIII, fibronectin, antithrombin III, alpha-1-antitrypsin, and prekallikrein. The antihemophilic factor protein which may be treated according to the method of this invention can be prepared according to any of the well-known, conventional techniques such as, for example, the technique disclosed in any of the following: Hershgold et al, J. Lab. and Clin. Med., 67, 23 - 32 (1966); and Mozen, Rev. Hematol., 1, 135 - 160 (1980). Fibronectin can be prepared by the method described by Engvall et al, Int. J. Cancer, 20, 2 (1977). Cohn Effluent II + III, which contains as major protein components antithrombin-III and alpha-1-antitrypsin, can be prepared by the alcohol fractionation method described in Cohn, U.S. Patent 2,390,074 and J. Amer. Chem. Soc., 68, 459 (1946). Prekallikrein can be prepared from plasma by, for example, first treating the

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starting plasma to lower the ionic strength, applying the plasma to a DEAE Sephadex® A-50 column, collecting and pooling the eluate containing prekallikrein peaks, contacting the prekallikrein pool with concanavalin A-Sepharose, and eluting purified prekallikrein from the concanavalin A-Sepharose.

It has been found that the fibrinogen (Factor I) content of the protein composition to be pasteurized is an important factor, the higher the fibrinogen content, the greater the amount of <sup>sugar or reduced sugar</sup> carbohydrate needed. The fibrinogen content of the protein composition should be no greater than about 60%, based on the weight of total protein, or no greater than 0.6% based on the weight of solution at, for example, 54% sucrose (weight to volume). In a preferred embodiment the protein composition should contain no greater than 40% fibrinogen, based on the weight of solution. If the amount of fibrinogen in the composition to be pasteurized exceeds the above limits and the amount of <sup>sugar or reduced sugar</sup> carbohydrate is not increased, the thermal stability imparted to the therapeutically active proteins by the <sup>sugar or reduced sugar</sup> ~~peptone~~ is substantially reduced or lost completely.

A protein composition having a fibrinogen content greater than 60% can be pasteurized in accordance with our method (1) if the concentration of fibrinogen in the solution is below 0.6%, preferably below 0.4%, or (2) if a protein capable of being stabilized during pasteurization such as albumin and the like is first added to the protein composition to lower its fibrinogen content to less than 60% (the added protein generally should have the characteristic of being easily separable from the initial protein composition if necessary; it may be also that the added protein is compatible with the intended therapeutic use of the initial protein composition and, thus, need not be removed therefrom), or (3) if at least about 5 parts of <sup>sugar or reduced sugar</sup> ~~carbohydrate~~ are used per part of fibrinogen.

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Important products of this invention include pasteurized aqueous mixtures<sup>of</sup> therapeutically active protein compositions which may contain or be free of sugar or reduced sugar and amino acid and those pasteurized protein compositions being free of sugar or reduced sugars and amino acid and water. Pharmaceutical preparations containing therapeutic amounts of a protein composition pasteurized in accordance with the present invention are also contemplated. Particular products of the invention include pasteurized compositions containing antihemophilic factor (Factor VIII), fibronectin, alpha-1-antitrypsin, antithrombin III and prekallikrein.

As mentioned above the pasteurized products of the invention may be incorporated into pharmaceutical preparations, which may be used for therapeutic purposes. However, the term "pharmaceutical preparation" is intended in a broader sense herein to include preparations containing a protein composition pasteurized in accordance with this invention used not only for therapeutic purposes, but also for reagent purposes as known in the art; for tissue culture wherein organisms such as viruses for the production of vaccines, interferon, and the like, are grown on plasma or on plasma fractions, e.g., Cohn Effluent II + III, Cohn Fraction IV, Cohn Fraction V, and so further; etc.

For any of the above uses it is advantageous that the protein composition be free of infective hepatitis as provided in the instant invention. The pharmaceutical preparation intended for therapeutic use should contain a therapeutic amount of a pasteurized protein composition, i.e., that amount necessary for preventative or curative health measures. If the pharmaceutical preparation is to be employed as a reagent, then it should contain reagent amounts of pasteurized protein composition. Similarly, when used in tissue culture or a culture medium the pasteurized protein composition should contain an amount of

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protein composition sufficient to obtain the desired growth. It should be obvious that protein compositions pasteurized in accordance with this invention will not contain infective amounts of viruses and other organisms which are inactivated under the pasteurization conditions.

The invention described above is demonstrated further by the following illustrative examples.

Example 1

Effect of Sugar Concentration on  
Stability of Antihemophilic Factor

This experiment was carried out to determine the effect of increasing concentrations of sugar on the stability of antihemophilic factor (also referred to as AHF or factor VIII) upon heating at pasteurization conditions. An antihemophilic factor concentrate (commercially available from Cutter Laboratories, Berkeley, CA as KOATE® brand of antihemophilic factor) was reconstituted in sterile water for injection ("WFI") to give samples having an  $A_{280}$  of 14.8, 7.05, 3.4, 1.8, and 0.98. To aliquots of each of the samples having the given protein concentration ( $A_{280}$ ) was added 0.8% (54% w/v, 45% w/w), 1.0 (62% w/v, 50% w/w), 1.2 (70% w/v, 55.6% w/w), and 1.5 (77% w/v, 59.9% w/w) g/ml of sucrose. Each of the samples was heated at pasteurization conditions of 10 hours at 60° C. It was observed that only samples containing 54% added sucrose and having an  $A_{280}$  of 14.8, 7.05, 3.4 and 1.8 became hazy. These data indicate that a sucrose concentration, representative of concentration of sugars and reduced sugars, of 54% (w/v) is the minimum concentration of sugar required to provide advantageous stability to heat of the thermally sensitive, therapeutically active proteins selected from AHF, fibronectin, antithrombin-III, alpha-1 antitrypsin and prekallikrein.

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Example 2

Effect of High Sugar Concentration and Amino Acid  
on Stability of Antihemophilic Factor

This experiment was carried out to further demonstrate the importance of increased sugar or reduced sugar concentrations combined with amino acid on the stability of AHF upon heating at pasteurization conditions. Test samples of the AHF concentrate described in experiment 1 above were reconstituted in sterile WFI. To each sample was added sodium citrate (0.1 M), glycine (0.3 M) and sodium chloride (0.15 M). Sucrose was added to the samples at a concentration of 1.2 g/ml (70% w/v, 55.6% w/w) and 0.8 g/ml (54% w/v, 45% w/w). The  $T_{580}$  (transmittance) of the samples was measured and used as the basis for determining the degree of protein (AHF) denaturation upon pasteurization, water reference  $T_{580}$  being 100%. Each of the samples was heated at pasteurization conditions for 10 hours at 60° C. The results are given below.

<u>Sample</u>	<u>Sucrose Concentration</u>	<u><math>T_{580}</math></u>	<u>% AHF Recovery*</u>
Test #1	1.2 g/ml	94 %	66.0 %
	0.8	68 %	23.8 %
Test #2	1.2	96 %	69.4 %
	0.8	86 %	38.5 %
Test #3	1.2	95 %	61.7 %
	0.8	79 %	51.4 %

\* Recovery determined by analysis for procoagulant activity by the methods of Langdell et al, J. Lab. Clin. Med., 41, 637 (1953) and Proctor et al, Am. J. Clin. Path., 36, 212 (1961).

It was observed that in each of the tests, # 1 - 3, recovery of AHF was greater with the higher concentration of sucrose, the average recovery of AHF being 65.7% with

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### Effect of 77% (w/v) Sugar Concentration and Amino Acid on Stability of Antihemophilic Factor

The experiment described in Example 2 above was repeated except that sucrose was added at a concentration of 1.5 g/ml (77% w/v, 59.9% w/w) in place of the concentration of 1.2 g/ml described in Example 2 to determine whether further improvement in AHF recovery could be achieved using sucrose concentrations greater than 1.2 g/ml. Although in several attempts VIII:C analysis was made difficult by the crystallization of sucrose during freezing and thawing, in one successful attempt using 1.5 g/ml, recovery was 80% for AHF prepared from an acid-chill effluent in 0.01 M sodium citrate, 0.3 M glycine and an  $A_{280}$  of 10.

### Effect of pH, High Sugar Concentration and Amino Acid on Recovery of Antihemophilic Factor

This experiment was carried out to determine the effect of pH on the recovery of AHF upon pasteurization and to

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illustrate the effect of the combination of higher sugar concentration and lower glycine concentration than was heretofore known. An antihemophilic factor (AHF, or factor VIII) solution was prepared by the method of Mozen, Rev. Hematol., 1, 135 - 160 (1980). To the solution so prepared was added 0.01 M sodium citrate and 0.3 M glycine and then the solution was diluted with WFI to an  $A_{280}$  of 25. Aliquots of this solution were taken and the pH of the aliquots was adjusted with sodium hydroxide or hydrochloric acid to the values shown in the table below. Aliquots with lysine added to give a solution 0.32 M in lysine and without lysine were treated with 1.2 g/ml of sucrose to provide test samples having a sucrose concentration of 70% w/v (55.6% w/w) based on starting solution. All aliquots were heated at pasteurization conditions for 10 hours at 60° C. Control aliquots were prepared as above but were held at 5° C during this time. Control and pasteurized samples were analyzed for recovery of procoagulant activity by the methods of Langdell et al, J. Lab. Clin. Med., 41, 637 (1953) and Proctor et al, Am. J. Clin. Path., 36, 212 (1961). The results are given below:

pH	% Recovery of AHF	
	0.32 M lysine	No lysine
6.55	100%	85%
6.9	93%	66%
7.2	86%	48%
7.6	62%	68%

These data indicate that carrying out the pasteurization at a pH in the range of 6.0 - 7.5, preferably 6.55, using as the heat stabilizer a combination of 0.3 M glycine and 0.32 M lysine with a sucrose concentration of 70% w/v (55.6%, w/w), affords advantageous stability to heat of AHF that was heretofore not known. Although the mechanism by which this improvement is achieved is not fully understood, the data suggests that pasteurization conditions at a pH that

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is compatible with AHF and also that is remote from the optimal pH at which proteolytic enzymes may operate to diminish AHF procoagulant activity may afford higher recovery of AHF.

Example 5  
Effect of Varying Amino Acid Concentration with  
High Sugar Concentration on Recovery of  
Antihemophilic Factor

This experiment was performed to further determine and characterize the effect of the combination of lower concentrations of amino acid, particularly the mixture of lysine and glycine, with higher sucrose concentrations than was known heretofore. An AHF solution was prepared by the method of Mozen, Rev. Hematol., 1, 135 - 160 (1980). To the solution so prepared there was added 0.005 M sodium citrate and 0.16 M glycine. Then, the solution was adjusted to pH 6.9 and diluted with WFI to an  $A_{280}$  of 21. Aliquots of this solution were taken and lysine concentration in the aliquots was varied. To the aliquots was added 1.2 g/ml of sucrose and the resulting solutions were heated at pasteurization conditions for 10 hours at 60° C. The results are given below:

<u>Lysine</u>	<u>% AHF recovery</u>
0	76.5%
0.04 M	76.5%
0.08 M	68.2%
0.16 M	80.6%
0.32 M	89.9%

These data indicate that the use of a combination of a mixture of 0.16 M glycine and as low as 0.04 M lysine with 1.2 g/ml (70% w/v, 55.6 w/w) of sucrose affords advantageous stability to heat of AHF that was heretofore not known.

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Example 6  
Effect of Varying Amino Acid Concentration with  
High Sugar Concentration on Recovery of  
Antihemophilic Factor

This experiment was carried out to determine the effects varying low concentrations of glycine or arginine or a combination thereof in the presence of an AHF solution having an  $A_{280}$  at 10.1 and containing 0.01 M of citrate and 1.2 g/ml of sucrose at pH adjusted to 7.18. The solutions were heated at pasteurization conditions for 10 hours at 60° C. The results are given below:

	<u>% AHF Recovery</u>
citrate only	41.1%
0.1 M glycine	45.0%
0.3 M glycine	61.3%
0.5 M glycine	75.4%
0.05 M arginine	64.2%
0.1 M arginine	71.0%
0.15 M glycine + 0.05 M arginine	61.5%

These data indicate that the use of as low a concentration as 0.3 M of glycine alone, or as low a concentration as 0.05 M arginine alone, or a concentration of 0.15 M glycine together with 0.05 M arginine, each in combination with 1.2 g/ml (70% w/v, 55.6% w/w) of sucrose, affords advantageous stability to heat of AHF that was heretofore not known.

Example 7  
Effect of High Sugar Concentration and  
Amino Acid on Recovery of Fibronectin Activity

This experiment was carried out to illustrate the effects of various combinations of heat stabilizing amino acids and sugars or sugar alcohols on fibronectin. Fibronectin was prepared by the method of Engvall et al, Int. J. Cancer,

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20, 2 (1977) using the gelatin-Sepharose® affinity medium method. Fibronectin was eluted from the affinity medium using 4 M urea and urea was removed by diafiltration. The resulting fibronectin was assayed and found to be greater than 95% pure. Samples were formulated and treated under pasteurization conditions for 10 hours at 60° C as set forth in the table below:

Run	Pasteurization Conditions (60° C, 10 hours)	Recovery of therapeutic activity	
		a Agglutination assay (%)	b Rat Liver slice assay (%)
A	57% sucrose (0.81 g/ml)	105	120
B	57% sucrose, 0.5 M arginine	118	60
C	0.5 M arginine	10	—
D	0.5 M lysine	5	—
E	control (no heat stabilizer)	<1	0
F	control, held at 5° C for 10 hours	100	100

a = Agglutination Assay described by Check et al in the  
J. Reticuloendothelial Soc., Vol. 25, pages 351-362 (1979).

b = Rat Liver Slice Assay described by Molnar et al in Biochemistry,  
Vol. 18, page 3909 (1979).

These data indicate that the use of a low concentration of amino acid and higher (57%) concentration of sucrose as stabilizer against heat under pasteurization conditions affords advantages which were not heretofore known.

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WHAT IS CLAIMED IS:

1. A method of pasteurizing a composition comprising a thermally sensitive, therapeutically active protein selected from the group consisting of antihemophilic factor (Factor VIII), fibronectin, antithrombin III, alpha-1-antitrypsin, and prekallikrein, which comprises -

(a) mixing the protein composition with from 0.04 to 0.8 M of at least one amino acid and a compound selected from the group consisting of sugars and reduced sugars in an aqueous medium, said compound being present in the mixture in an amount of about 54% to saturation, on a weight to volume basis, and

(b) heating the mixture at a temperature of about 60 - 75° C and a pH of about 5.5 - 8.0 for at least about 10 hours to pasteurize the protein composition and render it substantially free of infective hepatitis.

2. The method of claim 1 wherein the molecular weight of the compound is about 5000 or less.

3. The method of claim 1 wherein the pH of the mixture is about 6.0 - 7.5.

4. The method of claim 1 wherein the compound is sucrose.

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5. The method of claim 1 wherein the amino acid is selected from the group consisting of arginine, lysine and glycine.

6. The method of claim 1 wherein the amount of compound is sufficient to result in the retention of at least about 40% of the activity of the protein during pasteurization of the protein composition.

7. The method of claim 1 wherein the mixture is heated in Step (b) at a temperature of about 60° C.

8. The method of claim 1 which further includes <sup>at least</sup> one of the steps of removing said compound from the mixture resulting from Step (b), removing said amino acid from the mixture resulting from Step (b), and removing both said compound and said amino acid from the mixture resulting from Step (b).

9. The method of claim 8 wherein the compound and/or amino acid is removed from the mixture of Step (b) by subjecting the mixture to diafiltration.

10. The method of claim 8 wherein the compound and/or amino acid is removed from the mixture of Step (b) by subjecting the mixture to dialysis.

11. The method of claim 8 which further includes the step of removing water from the mixture of Step (b).

12. The method of claim 11 wherein water is removed from the mixture of Step (b) by subjecting the mixture to ultrafiltration.

13. The method of claim 11 wherein water is removed from the mixture of Step (b) by subjecting the mixture to freeze-drying.

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14. The method of claim 1 wherein the amount protein composition present in the mixture in Step (a) is from about 1 mg/ml to about 15 mg/ml based on total mixture, the amount of said amino acid present in the mixture in Step (a) is from about 0.04 M to about 0.8 M based on total mixture, and the amount of said compound present in the mixture in Step (a) is from about 0.8 g/ml to about 1.5 g/ml based on total mixture.

15. The method of claim 1 wherein said protein is selected from antihemophilic factor and fibronectin and is present in the mixture in Step (a) in the range of from about 1 mg/ml to about 15 mg/ml based on total mixture, and wherein said amino acid is selected from arginine, lysine and glycine and is present in the mixture in Step (a) in the range of from about 0.04 M to about 0.8 M based on total mixture.

16. The method of claim 15 wherein the mixture is heated in Step (b) at a temperature of about 60° C.

17. The method of claim 16 which further includes <sup>at least</sup> one of the steps of removing said compound from the mixture resulting from Step (b), removing said amino acid from the mixture resulting from Step (b), and removing both said compound and said amino acid from the mixture resulting from Step (b).

18. The method of claim 16 which further includes the step of removing water from the mixture of Step (b).

19. The method of claim 17 which further includes the step of removing water from the mixture of Step (b).

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