

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

3

4 GRO-A, and GRO-A personally)
and as surviving guardians of)
5 GRO-A, 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

16 TRANSCRIPT OF TESTIMONY OF
17 PETER FERNANDES

18

19

Before the Honorable

20

Anthony J. Metz, III, Judge

21

February 12 and February 13, 1997

22

23

24

25

26

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

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

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

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

MR. JACKSON: No.

MR. GOODELL: Are you planning on doing others?

MR. JACKSON: Yes.

MR. BARR: Your Honor, --

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

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

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

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

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

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
18
19
20
21
22
23
24
25

THE COURT: Is this 1001?

MR. JACKSON: Yes, Your Honor.

Additional pages from the lab notebook.

THE COURT: Are you going to offer 1001?

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

THE COURT: Any objection?

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

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
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
1

morning.

**WHEREUPON THE JURY WAS ADMONISHED AND
EXCUSED FROM THE COURTROOM**

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

8

9

10

DEPOSITION OF DR. FERNANDES RESUMED

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

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

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

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

Q. But material that Cutter was then manufacturing?

A. Was then manufacturing.

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 abstract 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
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

WHICH WAS ALL OF THE TESTIMONY GIVEN BY
PETER FERNANDES ON SAID DATES

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

18

19

20

21

22

23

24

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]

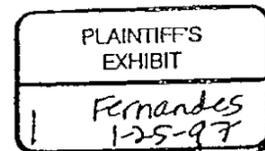
Experience

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.

1-1



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 IND and completion of Phase I Trials

1-2

Group Leader , Beta Interferon

March 1983 - May 1987 ³

- * 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, 'GAMIMMUNE'-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

PLAINTIFF'S EXHIBIT
999

PLAINTIFF'S EXHIBIT
3 Fernandes
1-25-97

3-1

TO: Bart Bradley

FROM: Liter Fernandes / John D. Kunkel - 25
Inventor Date

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

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

TITLE: Pasteurization of Albumin and Immune Serum Globulin 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, as 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 | Mechanical | 5. Drawing |
| 2. Title | | 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, etc acetyl tryptophan, etc. These materials bind to albumin and stabilize the whole protein against thermal denaturation.

Gamma globulin, unfortunately, 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

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.

111 4379

1000

CUTTER Laboratories, Inc.

Page No.

37

Record Book No. 2021

Continued from Page

Pasteurization of 5% MISG

3x50 vials of 2021-04 (5% 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 / 0.22µ absolute filter.

Apparently, this heating step can be utilized to suppress or inactivate 1966 or 1965 products against hepatitis contamination.

T ₅₈₀ in heated material	99.5%
T ₅₈₀ , 60°C, 15 hours	95.3%

PLAINTIFF'S EXHIBIT

4 Fernandes 6-25-97

Read & Understood

Graced by

10/25/78

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

GRO-C

111 2 079

Continued on Page

Date

10-25-78

2021

Research Record Book No. 2021
Continued from Page 37



Pasteurization expts (Continued)

PK	Sample Description	60°C, 10 hrs	Notes
2631	2634 - 15% MISC Inulin	yelled	2021-35 H pasteurized & filtered 2661
		yelled	
		yelled	
	0.5M dextrose 5% MISC	clear	
PK 1709	maltose 2% 1.5%	Milky	on filtration (v. difficult)
PK 2661	maltose 2%	clear	on filtration less milky & more clear

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

5% Albumin (autoclaved)	yelled
5% Albumin, 10% dextrose	v. clear
10% maltose	less clear

- ① Glycose linkers contribute to turbidity on heating
- ② Glycose better than maltose

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

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

2 x 20 ml 3 hrs 60°C - filtered 5°C overnight, pasteurized
 1 x 5 ml - filtered, pasteurized immediately
 1 x 20 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

PLAINTIFF'S EXHIBIT
1001

PLAINTIFFS EXHIBIT
5 Fernandes 1-2597

5-1

MIL 028889

CUTTER Laboratories, Inc.

Research Record Book No. 2021-~~4~~

Continued from Page X

Polio & Measles Potency in Source Plasma

BM Full Code	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 of 196

Measles Ratio

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

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

∴ 0.50
0.32

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

GRO-C

1111 21979

Continued on Page

Date

11/78

INT 503

5-2

CUTTER Laboratories, Inc

Page No.

Research Record Book No. 2021

Continued from Page



AA Analysis

Samples Submitted

Additional lots from same batch

2631
2634
2649
2660
2661
2662
2664
2577
262704
2705
2709

2632, 2633

2663

2680
2679
2710
2703

Effect of Caseinate, tryptophan on MISA pasteurization
Sample composition

5% MISA in
- 0.04M Caseinate
- 0.04M acetylated tryptophan
- 0.04M Cap + 0.04M trypt
- 0.04M Cap + 10% Maltose + 0.04M trypt
- 0.04M trypt + 10% Maltose
- 10% Maltose

All gelled solid

→ slightly gelatinous

LIBRARY

GRO-C

JUL 2 1975

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

Date

11/7/78

RET 11/7/78

Continued on Page

5-3

MIL 029892

CUTTER Laboratories, Inc

42

Research Record Book No. 2021

Page No. []

Continued from Page []

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

Sucrose 2.78%

LIBRARY

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

GRO-C

JUL 2 1978

Continued on Page []

Date 12/11/78

INITIALS []

54

MIL 028893

CUTTER Laboratories, Inc.

Page No.

Research Record Book No. 2021-43

Continued from Page

Protein Content of desalted MISC = 6.29%
81ml 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 + 1M HCl 50 ml
- ⑤ in 15% Maltose 100 ml
- ⑥ in 50% Maltose 100 ml
- ⑦ in 0.3M dly / 0.45% Li 150 ml

12/7/78

230 ml of 6% MISC (low salt)

175

Added 40gms sucrose

Final volume 300 ml 325 ml

Sucrose conc? 5.2%

Pasteurized for 10 hrs at 60°C - particles

Protein conc? 1.16%

Dialyzed against WFI over weekend. Slight pptn.

Protein 1.16%

Concentrated using XM-100A to 6% added Maltose

& figure. sterile filtered

Filled 16 x 10 ml

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

LIBRARY

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

GRO-C

JUL 21 1979

Continued on Page

Date

12/2/78

INIT

5-5

MIL 028894

CUTTER Laboratories, Inc

Research Record Book No. 2021

Page No.

44

Continued from Page

2021-44

Final Bulk Samples on Rabies V/GA - see pg 43 for EPTT

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 attempted with sucrose (1.5gms) 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:

Jane J. Rig

4/8/79

PLAINTIFFS EXHIBIT

6 Fernandes 1-25-97

GRO-C

LIBRARY

JUL 2 1979

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

Continued on Page

Date

12/14/78

PLAINTIFF'S EXHIBIT

971

MIL 028895

8

PLAINTIFF'S EXHIBIT
7 Fernandes
1-25-97

7-1

TO: Albert Bradley

12/14/78

FROM: GRO-C

SUBJECT: Disclosure
(Conception or invention)

WITNESS: GRO-C 12/10/78
Read & Understood

WITNESS: GRO-C 12/14/78
Read & Understood

TITLE: Pasteurization of Fibrinogen, Factor VIII, Factor IX (Kongie), Gamma Globulin, etc. Plasma
etc. 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! NO! 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
5. Drawing
6. That drawing shows
7. How it works
8. Your signature and two witnesses who understand your disclosure

Mechanical

PLAINTIFF'S EXHIBIT
291

DISCLOSURE:

12/14/78

This disclosure is for a wider application than the one entitled "Pasteurization of defatted albumin and human serum globulin 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 particle 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

111 4127

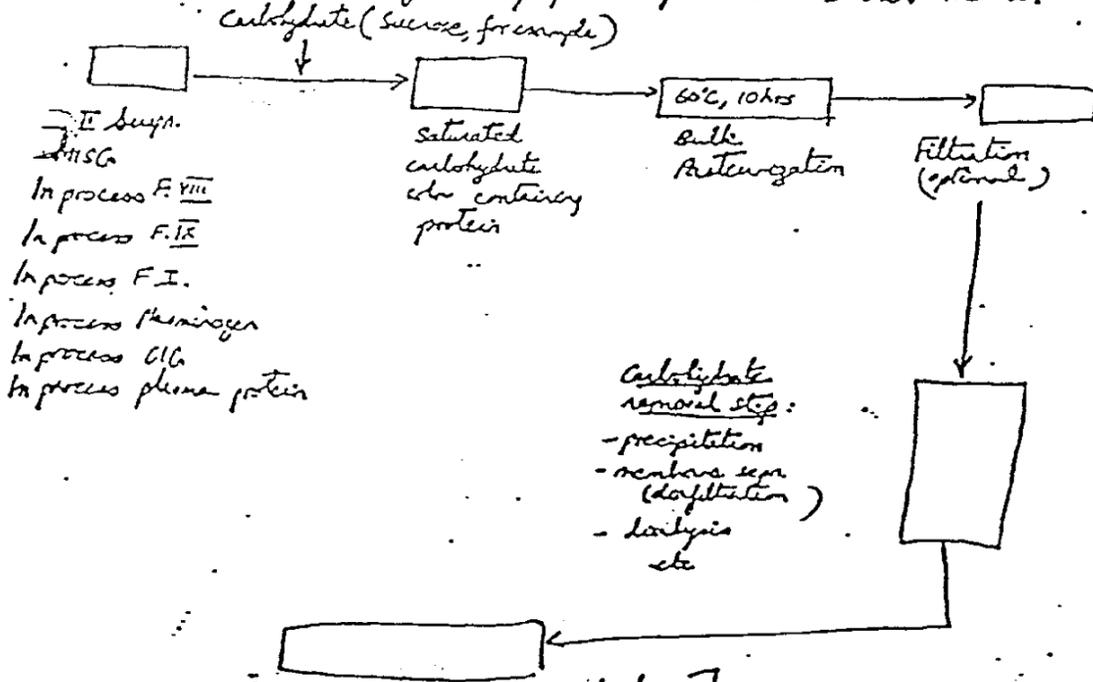
fragile proteins during pasteurization

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% loss in activity of *prothrombin* after heating to 50°C for 48 hours. The paper is

A schematic of our proposed process is shown below:



Further processing & final container
 Since this is a bulk pasteurization step, all processing on the upstream side would have to be done in a hepatitis free area.
 It should be noted that all carbohydrates could theoretically be used to protect the protein during pasteurization

111 4328

Research Record Book No. 2001

Continued from Page _____

Pasteurization of AHF

58 57.89 gm (-60ml) of AHF sterile scrap C-1057
 Activity 27 units/ml Drawed and 50 g sucrose was
 added

Total vol 86ml

Pasteurized from 9:30 pm 2/18 to 7:20 am Result: Gelatin

Test 2/19

4 vials of M 5368 AHF
 Reconstituted in 1ml ea. WFI

1 vial Karyne in 20ml

- Vial 1 + 3g sucrose
- Vial 2 + 5g sucrose
- Vial 3 + 7g sucrose
- Vial 4 + 10g sucrose

Vial ③ in 5g suc
 ④ in 10g suc
 mixed at 14:00

Testing started:	1	2	3	4	5	6
9 am clear	clear	clear	same as base		clear	clear
9:45 am					clear	clear
11 pm	clear		clear	clear	clear	clear
1 gm	C	L	E	A	R	
2 pm						
3 pm						
4:30 pm						
7:30 am		slight op.	less op.	clear	clear	
A-580 post heat	0.049	0.030	0.018	0.030	0.018	0.03

GRO-C

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

Continued on Page _____

Date

2/18/78

PLAINTIFF'S EXHIBIT
 972

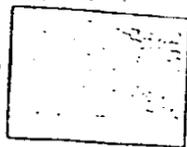
PLAINTIFF'S EXHIBIT
 8 Fernandes
 1-25-97

MIL 028896

CUTTER Laboratories, Inc

Research Record Book No. 2021 ³⁰

Continued from Page _____



Using C-1051 material:

A	10ml + 3g
B	+ 5g
C	+ 10g
D	+ 0g
E	Kept by self

8:15am inserted A B C D
 8:25am inserted E
 8:40pm ← Collected →

Results of source pasteurized MISG - Final formulation
 5% MISG, 10% maltose, 0.1M glycine

GFC	2 Vol	2 Dimer	2 Monomer	2 C Monomer		
	6.7%	7.4%	75.9%	0.7%		
SISPEE	H ₂ L ₂	H ₂ L	H ₂	HL	H	L
①	8	7.6	9.6	11.9	36.7	26.3
②	6.5	7.7	10.1	11.2	38.3	25.8

Antibody potency assays:

	Monomer titer	Diphtheria (units/ml)
Pasteurized MISG	0.3	0.8
AC	8.4 mg/CHSD	>1<2

Past. MISG 1948-98 Control
 1948-99 Past. (passed safety)

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

GRO-C

JUL 2 1978

Continued on Page _____

Date

12/18/78

Page

8-2

CUTTER Laboratories, Inc.

Research Record Book No. 2021

Continued from Page

Pasteurization studies on Korte

A vial of M 5368 Korte, lyophilized were reconstituted in 10 ml each with of WFI

Vial #	Sucrose added, g	60°C/22 1/2 hours	Past A5 T580	% in Sucrose
1	3	clear	97.218	
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	Past T580 2
5	5	clear	97.04 2
6	10	clear	98.21 7

LIBRARY

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

GRO-C

JUL 2 1978

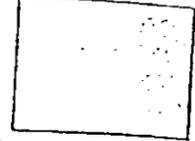
Continued on Page

Date

8-3

Research Record Book No. 2021

Continued from Page



Pasteurization of ISG

150 gm R.I., 84 7213 resuspended in WFI Total volume ~ 500 ml.

Filtered 500 ml thru 0.8 μ Nalgene. Protein 9.71%

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 (calcd) 0.13 gm protein

Ex. 2025 reconstituted in 10 ml WFI cu.

Total volume 50 ml.

Added 40 gm sucrose.

Pasteurized 10 hrs @ 60°C

Removal of sucrose

ISG

The pasteurized material is dialyzed against WFI for 24 hr

precipitated material present in dialysis bags.

Probable reasons: (A) alcohol in R.I. parts
(B) low salt conditions
- precipitation of cryoglobulins.
Discontinued run.

AHF

The pasteurized material was dialyzed against f.c. AHF buffer for 12 hrs.

0.3M Glycerol

0.15M NaCl

0.01M sod citrate $\cdot 2H_2O$ (FW 294)

~ 50 ml in dialysis bag in 20L of above buffer.

initial feed A₂₀₀ (bottle) A₂₀₀ = 0.7 x 25

A₂₀₀ in dialyzed material = 0.33 x 25

Vol of dialyzed material ~ 200 ml

2021-48A

2021-48B

LIBRARY REC'D

GRO-C

JUL 2 1979

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

Continued on Page

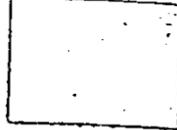
Date 1/2 / 79

JUL 2 1979

74

Research Record Book No. 2021-

Continued from Page 48



Coagulation results

Feed 27 unit/ml at $A_{220} = 0.7425 = 17.5$ units

Pasteurized Material 290-488 2.5 unit/ml @ $A_{220} = 0.16 \times 25 = 4$ units

Feed $27/17.5 = 1.54$ units/ A_{220}

Pasteurized material $2.8/4 = 0.7$ units/ A_{220}

= 45.4% recovery.

Pasteurization of Defatted Albumin

2021-49

62.5 gm of Albumin (acetone dried powder) suspended in 624 0.15M NaCl. \approx 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 stored for 1 hour. Centrifuged and filtered thru Whatman #54, 0.5 μ , 0.45 μ , 0.2 μ . (Filtered only 200ml). Raised pH to 7.04 and froze in -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. Will carry out SDS PAGE ASSAYS

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

GRO-C

JUL 2 1979

Continued on Page

Date

1/4/79

7-5

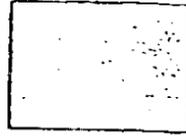
MIL 028900

CUTTER Laboratories, Inc.

Page No.

Research Record Book No. 2021

Continued from Page



CAE Analyses on AHF

		Fibr.	BF66
1	Albumin		
2	Albumin		
3	M 3094	32.7%	67.2%
4	M 3094	33.7%	66.2%
5	C-1051	63.3%	36.6%
6	C-1051	61.7%	38.2%
7	Paul Hys sample		
8			

Effect of Fibrinogen content

1 vial of K4724 Paragen (Exp 15 Oct 1974) was reconstituted and saturated with sucrose (5gms/10ml). Gelation occurred with 30 mins. @ 60C.

Paragen additives

- 1gm Fibrinogen
- 0.92gm Sodium citrate
- 2.5gm dextrose
- 60mg glycine
- pH adjusted with HCl/NaOH
- No preservatives

reconstituted in 50ml WFI

LIBRARY

1979

GRO-C

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

Continued on Page

Date

1/5/79

7-6

MIL 028901

Research Record Book No. 2021
Continued from Page _____

Page No.

Effect of Fibrinogen content on gelation time for AHF

2 vials of Koste M3094 Reconstituted in 20 ml WFI
1 vial of Parogen K4724 Reconstituted in 50 ml

Sample #	Ant. of AHF (μ l. increase)	Ant. of Fibrinogen (μ l. increase)	Ant. removed for CAE	Ant. heated 60°C	Gelation Time
1	6 ml	0	1 ml		
2	5 ml	1 ml	1 ml	4 ml	
3	4 ml	2 ml	1 ml	4 ml	3.7 hrs
4	2 ml	4 ml	1 ml	4 ml	1.63 hrs
5	1 ml	5 ml	1 ml	4 ml	48 min
6	0 ml	6 ml	1 ml	4 ml	17 min

CRE Analysis

Sample #	% β 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 ml of Albumin
Albumin standard

Albumin

100%
100%

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

GRO-C

Continued on Page _____

Date

1/8/77

PLAINTIFF'S
EXHIBIT
973

PLAINTIFF'S
EXHIBIT
9 Fernandes
1-25-77

MIL 028902

CUTTER Laboratories, Inc.

Page No.

52

Research Record Book No. 2021

Continued from Page _____

Pasteurization of Konjine

Konjine M 5542 (Exp. 19 July 78) reconstituted in 20 ml.
 Served 2 ml for CAE, protein. Served 8 ml for assay (2021-52A).

Pasteurized from 10:20 am to 8:30 pm @ 60°C.

Volume = 4 ml.

Froze 2 ml for assays (2021-52B)

Dialyzed 12 ml against 15L of f.c. buffer:

2. 0.0925M NaCl (5.4 g/L)

0.0525M sodium citrate (15.4 g/L) pH 7.3.

from 8:30 pm 1/2 to 8 am 1/9

Dialyzed volume 43 ml. (2021-52C)

2021-52A

A₂₈₀ = 1.116 x 25 = 27.9 A₂₈₀ units.

2021-52C

A₂₈₀ = 0.2 x 25 = 5 A₂₈₀ units

2021-52B

Raw RBD Coag data

1X unit

14.3 units

34 units/ml

0

0

Inhibitory

0.005

NAPIT ↑

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

GRO-C

JUL 2 1979

Continued on Page _____

Date

1/9/79

[Signature]

MIL 028903

CUTTER Laboratories, Inc.

Page No.

Research Record Book No. 2021

Continued from Page

Pasteurization of AHF

4 vials of Koate M 3094 reconstituted in 40 ml.
 Removed 2.5 x 2 ml, 1 x 1 ml as control samples.
 Volume remaining 34 ml. Added 28g. sucrose.
 Volume after sucrose added: 48 ml
 Control volume = 18 ml left @ 5°C for entire period.
 Balance: 30 ml pasteurized begin 9am + 10 min
 25 ml dialyzed

vs. 3M glycine
 15M NaCl
 0.1M Sodium Citrate
 1% dextrose

15L

also dialyzed (in same tank) 11. ml control
 volume after dialysis

	A ₅₈₀ *	A ₅₈₀ (0.25)
Control 43 ml	.024	.145 x 25 = 3.625
pasteurized 58 ml	.115	.098 x 25 = 2.45
feed material		.085 x 25 = 2.17

Note: blood spots in buffer. On outside of bag; it could not go in. (454 ml bag)
 Control vial. (could not go in 454 ml bag)

Submitted to Coagulation Lab for JUL, VIII C: WF

- 2021-52A Koate reconst. frozen no treatment
- 2021-52B Koate + sucrose at 5°C 10 hr.
- 2021-52C Koate + sucrose at 60°C 10 hr.
- 2021-52D B dialyzed 13 hr
- 2021-52E C dialyzed 13 hr

* A₅₈₀ taken after freezing + thawing

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

GRO-C

JUL 2 1979

Continued on Page 5A

Date 1/10/79

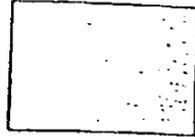
NOTE

CUTTER Laboratories, Inc.

Page No.

Research Record Book No. 2021-54

Continued from Page 53



~~282 P. 314~~

~~VS: .37M piperine
.15M NaCl
.017M Sodium Citrate~~

end 7:10 pm

Results from AHF Pasteurization Expt.

Raw data from	Coag. R & D Lab:	
2021-53A	26.6 \bar{v} units/ml	2
-53B	17.3	3
-53C	12.5	5
-53D	5.1	3
-53E	4.6	3

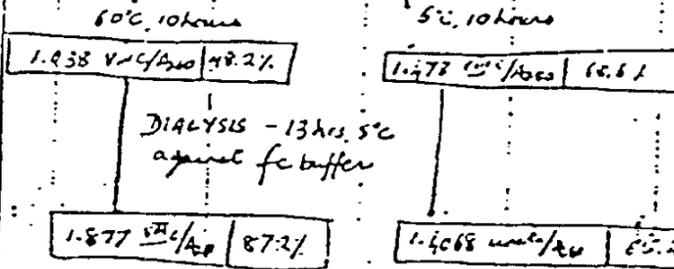
Specific Activity	\bar{v} units/ml
2.152	100%
1.478	
1.039	
1.406	
1.877	

Reassay

id. 1.355 \bar{v} /ml
act: 0.16 \bar{v} /ml
copy
62.5%

Feed 2.152 \bar{v} units/ml 100%

↓ sucrose



On reassay:
2021-53A 22.2 \bar{v} /ml
2021-53E 2 \bar{v} /ml

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

GRO-C

LIBRARY

JUL 2 1979

Continued on Page

Date

1/5/79

ENT

Research Record Book No. 2021

Continued from Page

Pasteurization of Kongre

AHF Buffer

- 0.3M Cl₂ine
- 0.15M NaCl
- 0.01M Sod. Citrate
- 1% Dextrose

Kongre Buffer

- 0.04M Sod. Citrate
- 0.08M NaCl

Discussion with Bob Louis

Pasteurization @ 60°C, 10 hrs does not necessarily destroy antigenicity - like 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 cells 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. Dialyzed 50 ml against f.c. buffer for 4 hrs. & aborted.

- 2021-56A - feed material K7331, Kongre.
- 2021-56P - post dialyzed material (vol. 200 ml)

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

GRO-C

LIBRARY

JUL 2 1979

Continued on Page

Date 1/5/77

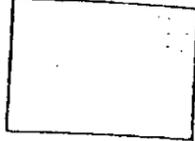
LIB

CUTTER Laboratories, Inc.

Page No.

Research Record Book No. 2021

Continued from Page _____



Dec 19 55 for Koigia pasteurization study

CAE of various Kosta lots

	Batch	Albumin	Bilirubin	Fibrinogen
M 2835	(1)		66.1	33.9
	(2)		63.8	36.3
	Mean		64.9	35.1
PR 2552	(3)	11.7	36.4	51.9
	(4)	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
M 3143	(7)		56.3	43.1
	(8)		61.9	38.0
	Mean		59.4	40.7

LIBRARY

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

GRO-C

JUL 2 1979

Continued on Page _____

Date 1/16/79

[Signature]

MIL 028907

CUTTER Laboratories, Inc.

Page No.

Research Record Book No. 2021

Continued from Page

Amnt of glycine required (1.6M) = 120 gm/L

Amnt of solution = 145-67 = 212 ml = 0.212L

Amnt of glycine 25.4 gm

This was added to the soln in the 5°C. Reaction 45 mins.

Removed 15ml (2021-578)

Concentrate to ~100ml. Decanted to ~100ml. replacement aged for buffer and froze 2021-57C P (IF problems)

MISG - C⁴ Expt

2021-58

Used BPR

1-24-77

Defatted Albumin polymerization (1948-92)

Results

	Final Monomer
1. Defat Albumin + 8 gm sucrose - (no salt) 10ml	27.58%
2. 10ml defat Alb + 2g sucrose (part.)	17.8%
3. 10ml defat Alb + 4g sucrose (part.)	27.5%
4. 10ml defat Alb + 6g sucrose (part.)	34.4%
5. 10ml defat Alb + 8g sucrose (part.)	37.18%

LIBRARY

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

GRO-C

JUL 2 1975

Continued on Page

Date

1977

INT 22

MIL 028909

CUTTER Laboratories, Inc.

Page No.

59

Research Record Book No. 2021

Continued from Page

Hepatitis Antigen study on Korte

Design of experiment

rate used: M2835 (Nov 75) 19.5 units/ml, 0.0079 gm/ml (label)

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 show 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.

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

GRO-C

LIBRARY

JUL 2 1979

Continued on Page

Date

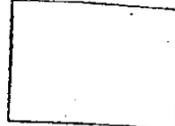
1-23-79

MIT

MIL 028910

Research Record Book No. 1021

Continued from Page _____



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 A280₁₅₀
- ② Add sodium citrate 2.94 g/L
- ③ Add solid Glycine 120 g/liter initial T=10°C
- ④ React 1 hour
- ⑤ CF₂ @ 5°C

supernatant 4x4 ml Label 2021-61B - Measure A280₁₅₀
paste weight: Label 2021-61C

- ⑥ Difilter against 0.0M sodium citrate, 0.1M glycine for 2 replacements and 0.1M NaCl

<u>Retention:</u> Initial level	glycine	sodium citrate
0	1.6M	0.02M
1 replacement	0.58M + .067	.0072 + .006 = 0.013
2 replacements	= 0.647M	
	0.216 + 0.084	.0027 + .008
	= 0.31 M	= 0.011 M

Concentrate to A₂₈₀ = 31
save 60 ml

15 x 4 ml

2021-61D

LIBRARY-144

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

GRO-C

JUL 2 1979

Continued on Page _____

Date 1-29-79

INT 882

Research Record Book No. 2021

Continued from Page

2am 1/30 Part salt AHF (6M-7364) pH 6.94

Tot 1.75L Round 6x4ml 2021-61A

Balance 1.925L, -9°C

Asso. = 0.522

Total feed to 8.05

Titrate required 2.94 x 1.925
Glycine (1.6M) = 120 x 1.925

5.66g - Mixed for 5min.
231g 1 hour. Temp dropped to
4°C. Ran in + 5°C Room

Glycine addition completed 2:30am.

Centrifuged @ 5000 rpm for 30 min. using 1L bottles @ 5°C

Collected 2026ml of supernate.

Filled 6x4ml 2021-67B

2000ml
Dialyzed with Amicon 1 1/2 hollow fiber cartridge HIP10 against
0.1M glycine
0.01M succinate
0.15M NaCl

of this rate at end = 2.4 L/hr

Dialysis start ~4:30am

FR ~750ml/hr (Initial)

Asso. of succinate after replacement 1.25 x 0.24 = 6 units

Dialysis stopped
UF started
UF stopped

7:10am Volume dialyzed = 3L

7:10am
8:05am Volume concentrated to 540ml

Final Asso. = 0.80 = 20 units

10ml - assay ~15x4ml

saved 6x50ml

Vial 230ml

195g sucrose preincubated 2.5min to 6:45pm
SAMPLE AFTER 7AM. NOT DIAL = 2021-625 (pH 6.79)
Dialyzed vs 60L BUFFER (.3M GLYCINE, .15M NaCl, .01M Na Citrate)

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

GRO-C

LIBRARY

Continued on Page

Date

1-30-71

JUL 2 1975

INT 023

CUTTER Laboratories, Inc.

Research Record Book No. 2011

Continued from Page _____



Dialyzed volume = 1060 ml

60 ml
Removal of serum
(2021-62F)

1000 ml Concentration start 8:00 am.
ultrafiltered to 178 ml.
Concentration completed 7:10 pm.

Assay 2021-62F

The ultrafiltered samples are designated 2021-62G

Filled 15 x 4 ml + 10 x 10 ml + 1 x 20 ml

CAE Assays

2021-61A Post salt soln.

Alb	B	Fib
39.7	23.2	37
40.4	7.8	41.7
32.7	23.2	39.1
Mean 39.3	21.4	39.3

2021-61D Post Glycine/d/d/j/s/step

45.4	22.2	32.2
49	10.3	31.7
47.5	10.9	31.6
Mean 46.9	21.1	31.8

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

GRO-C

Continued on Page _____

Date

31/79

JUL 2 1979

CUTTER Laboratories, Inc.

Research Record Book No. 2021

Continued from Page _____

Assay results on 2021-61 *1 assay (15/79)*

Step	2021-61A	Volume	A ₂₈₀	YIII C / ml	Total YIII C	YIII C / ml	Total
<i>Past salt step: Alpine super UF</i>	2021-61A	1.825 L	5.05	3.6	6930	3	5725
	2021-61B	2.026 L	6.525	3.3	6686	3.2	6483
	2021-61D	547 ml (corrected) ($\frac{520 \times 2.026}{L}$)	22.35	8.8	4813	13.8	7549
<i>Dialysate</i>	2021-61D	200 ml	20.35	8.8	1760	13.8	2760
	2021-61F	1060 ml	5	1.1	1166	1.6	1696
<i>Post concentration</i>		189 ml (corrected) ($\frac{178 \times 1.16}{L}$)	23.77	4.5	851	7.8	857 1474

Corrected analyses using all the 2021-61 material

	Corrected	YIII C	Total
2021-61D	547 ml	4813	7549
2021-61F	2899 ml	3189	3189
2021-61G	516.9 ml	2326	2326
		4638	4638
			7549

Recovery across pasteurization step

$$\frac{YIII C}{YIII C} = \frac{3189}{4813} \times 100 = 66.25\%$$

$$\frac{YIII C}{YIII C} = \frac{2638}{739} \times 100 = 61.44\%$$

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

GRO-C

Continued on Page _____

Date 4/5/79

JUL 2 1979

Research Record Book No. 2021

Continued from Page

16-7-55
 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 soil for assay. 9:45 am to 1:30 pm
 supernatant vol 17 ml
 1 20 ml vol of M3143 reconstituted w/ 20 ml fibrinogen chr (from lyophilized Fibrinogen bottle #4661. Added 1.7g sucrose. Rinsed soil sample. Heated 60°C for 10.50 am. Cellulose by 1 pm. Fused at 15,000 rpm, 30 min. Supernatant vol 14 ml. Sampled.

	Albumin	β 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 \bar{v}_{111}^c
 PR 2552 feed 2021-91A
 PR 2552 treated 2021-91B

Continuation of results on C vs P MISL from Pg 84

SIDS PAGE

	22 drier	H ₂ L ₂	H ₂ L	H ₂	HL	H	L
2163-31P	1.4	3.3	3.4	3	7.4	53.7	27.3
2163-31C	0	3.2	4.6	5	8.1	52.5	26.6

I affirm that the above is a true record of work done by me GRO-C
 Date 5/10/74 JUL 21 1974

PLAINTIFF'S EXHIBIT
 974

PLAINTIFF'S EXHIBIT
 10 Fernandes
 1-25-97

MIL 028942

Cutter Laboratories, Inc
 MEMORANDUM

TO: J. Gihlin
 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

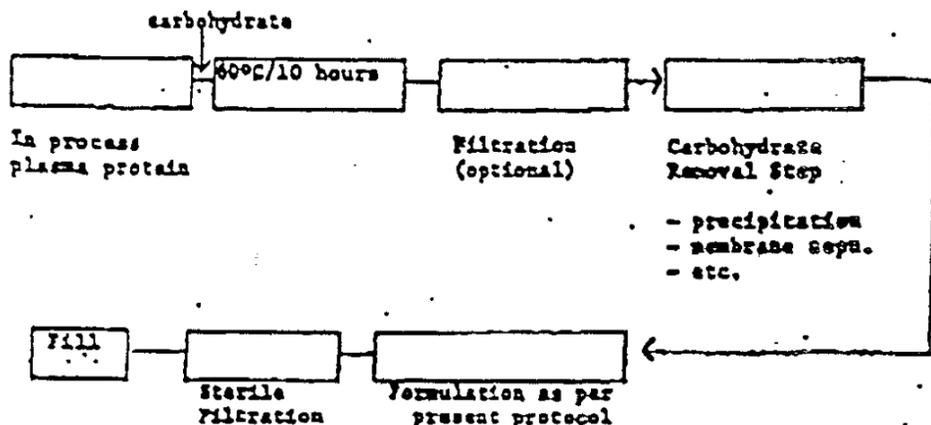
PATENT, TRADEMARK
 & LICENSING DEPT.

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
 11 GRO-C

MFK 001930

EX. 625 09691

PLAINTIFF'S
 EXHIBIT
 975

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 33% loss in activity. His work was not concerned with the preparation of clinical material.

Our results to date are shown below:

I. MISC (ISC) - Pasteurized 60°C, 10 hours in saturated sucrose

Test	Observed	Reference Value		
AC activity	8.4mg/CH50	Succ. MLT 3mg/CH50		
GFC-gel permeation chromatography, X		PP Ins Mean Values		
		in 0.1M Gly,	in 30% Maltose,	
		0.45% NaCl	0.1% Glycerol	
	> 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 ₁ L ₁	7.4		
	H ₂ L ₁	7.6		
	H ₂	9.9		
	KI	11.6		
	L	26.1		
Antibody Potency		PK 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.

MFK 001931

09692

11-2

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>Iβ Globulin</u>	<u>I Fibrinogen</u>	<u>Gelation Time, hours</u>
1	6.6	93.4	0.28
2	14.1	85.9	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® (OC096) resulted in a recovery of 45% VIII:C activity with a specific activity of 0.7 VIII:C units/A₂₈₀. VIII:K₁ 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

MFK 001932

09693

11-3

10/31/70

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.

PLAINTIFF'S
EXHIBIT
13 Fernandes
1-25-97

PLAINTIFF'S
EXHIBIT
23 Mosen
8-15-95

111 6019

23-1

PLAINTIFF'S
EXHIBIT
361

CL-370

DEP 063005

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;

DEP 063006

111 6020

CL-37D

23-2

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.

DEP 063007

The thermal instability of the aforementioned proteins renders them unpasteurizable. Therapeutically active

CL-37D

23-3

111 6021

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

111 6022

234

CL-37D

DEP 063008

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

CL-37D

DEP 063009

23-5

111 6023

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

111 6024

CL-37D

DEP 063010

23-6

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.

DEP 063011

The primary advantage of the invention is the availability of thermally stable and pasteurized therapeutically active protein compositions, which heretofore have been unknown

CL-37D

23-7

111 6025

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 ~~protein~~ 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, melezitose, and so forth. Exemplary of reduced sugars included within the purview of the invention are erythritol, ribitol, xylitol, sorbitol, mannitol, etc. ^{* below} 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

23-9

111 6027

CL-37D

* 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 ^{sugar or reduced sugar} ~~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 -

111 6428

CL-37D

23-10

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

CL-37D

23-11
DEP 063015

111 6029

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

CL-37D

23-12

DEP 063016

111 6030

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

CL-37D

23-13

DEP 063017

111 6031

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 ^{sugar or reduced sugar} 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 ^{sugar or reduced sugar} carbohydrate is not increased, the thermal stability imparted to the therapeutically active proteins by the ^{sugar or reduced sugar} ~~peptide~~ 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 ^{sugar or reduced sugar} ~~carbohydrate~~ are used per part of fibrinogen.

CL-37D

23-14

DEP 063013

111 6932

Important products of this invention include pasteurized aqueous mixtures^{of} 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

CL-37D

23-15

DEP 0630!9

111 6033

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.

111 6034

23-16

CL-37D

DEP 063020

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 T₅₈₀ (transmittance) of the samples was measured and used as the basis for determining the degree of protein (AHF) denaturation upon pasteurization, water reference T₅₈₀ 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>T₅₈₀</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

111 6035

CL-37D

23-17

DEP 063021

1.2 g/ml of sucrose and 37.9% with 0.8 g/ml of sucrose. The average T_{580} of 95% with the use of 1.2 g/ml of sucrose compared with the average T_{580} of about 78% with the use of 0.8 g/ml of sucrose gave evidence that a marked decrease in denaturation of AHF under the pasteurization conditions occurred with the use of the higher than with the lower sucrose concentration, each in combination with the glycine concentration of 0.3 M. These data indicate that the use of higher concentrations of sucrose, representative of concentration of sugars and reduced sugars, in combination with a lower concentration of glycine as the amino acid stabilizer against heat than was heretofore known, provides for advantageous recovery of AHF upon heating at pasteurization conditions for 10 hours at 60° C.

Example 3

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.

How was the acid-chill effluent prepared? Reference - 7.

Abbrev. of 12.11.1.1. v-5?

Example 4

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

CL-37D

23-18

DEP 063022

111 6036

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

111 6031

CL-37D

23-19

DEP 063023

is compatible with AHF and also that is remote from the optional 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.

CL-37D

23-20

DEP 063024

111 6038

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,

CL-37D

23-21

DEP 063025

111 6039

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.

111 6040

23-22

CL-37D

DEP 063026

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.

111 6441

23-23

CL-37D

DEP 063027

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 ^{at least} 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.

CL-37D

23-24

DEP 063023

111 6042

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 ^{at least} 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).

111 6043

23-25

CL-37D

DEP 063029