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CASE NO. CRIM 24552

SUPREME COURT OF THE STATE OF CALIFORNIA

THE PEOPLE OF THE STATE OF CALIFORNIA, PLAINTIFF, -VS- KEVIN COOPER, DEFENDANT.	SUPERIOR COURT NO. CR-72787 MOTIONS

APPEAL FROM THE SUPERIOR COURT OF SAN DIEGO COUNTY

HONORABLE RICHARD C. GARNER, JUDGE PRESIDING

REPORTERS' TRANSCRIPT ON APPEAL

APPEARANCES:

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OFFICIAL REPORTERS

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SUPERIOR COURT OF THE STATE OF CALIFORNIA

FOR THE COUNTY OF SAN BERNARDINO

THE PEOPLE OF THE STATE OF CALIFORNIA, Plaintiff, vs. KEVIN COOPER, Defendant.	NO. OCR-9319 CR-72787 VOLUME 18 Pgs. 1449 thru 1566, inc.

REPORTERS' DAILY TRANSCRIPT

BEFORE HONORABLE RICHARD C. GARNER, JUDGE

DEPARTMENT 10 - SAN BERNARDINO, CALIFORNIA

Monday, April 30, 1984

APPEARANCES :

For the People:

DENNIS KOTTMEIER
District Attorney

DENNIS KOTTMEIER
District Attorney
BY: JOHN P. KOCHIS
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I N D E X

EXHIBITS	FOR I.D.	IN EVIDENCE
7 - Vitae	1453	*
K-1 - Chart	1473	*
K-3 - Chart	1475	*
K-9 - Chart	1539	*

SAN BERNARDINO, CALIFORNIA, MONDAY, APRIL 30, 1984; 9:45 A.M.

1-1 DEPARTMENT NO. 10 HON. RICHARD C. GARNER, JUDGE

APPEARANCES:

The defendant with his Counsel, DAVID NEGUS, Deputy Public Defender of San Bernardino County; DENNIS KOTTMEIER, District Attorney of San Bernardino County, and JOHN P. KOCHIS, Deputy District Attorney of San Bernardino County, representing the People of the State of California.

(Leonard D. Gunn, C.S.R., Official Reporter, C-1109, Judith L. Morris, C.S.R., Official Reporter, C-2400)

THE COURT: Good morning, Mr. Negus, Mr. Kochis, everybody.

Mr. Cooper is present with counsel, all counsel.

We were to have a new witness this morning.

MR. KOCHIS: That's correct, your Honor. There are two procedural matters Mr. Negus and I believe should be discussed before I call Dr. Sensabaugh. He is our first witness this morning.

I believe Mr. Negus wants to take the lead in that.

MR. NEGUS: I would ask the Court to ask the Sheriff's Department to remove the manacles from Mr. Cooper's feet. Mr. Cooper has managed to get through approximately

1-2

60 or 70 court appearances without them.

THE COURT: I'm inclined to have that done. Is there some reason we can't do that? Does the Sheriff's Department have a reason?

MR. KOCHIS: I think they would like to speak to that, your Honor. They are concerned.

DEPUTY McCARVILLE: I'm Senior Deputy McCarville.

The problem we have with this courthouse is we have to bring him into the hallway to bring him into this courtroom. There is no way we can go through the back. We have to bring him up the stairs between 8 and 9 and bring him here.

THE COURT: Let's take them off in the courtroom.

DEPUTY McCARVILLE: Okay.

MR. KOCHIS: There is a problem in transporting Mr. Cooper into this courtroom. There is apparently no way for the Sheriff's Office to bring Mr. Cooper down the hallway in back of the courtroom and they have to bring him, I believe, up the elevator and then out a short distance in the hallway with the general public.

They are very concerned about that for his safety and the safety of the public.

THE COURT: The first thing I discovered when I got here this morning, unfortunately, is that that is the circumstance here.

DEPUTY McCARVILLE: Yes, sir.

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THE COURT: Anything further?

They are used to doing that. They have been doing it here for many years. They have had more difficult cases than this one as far as security is concerned.

DEPUTY McCARVILLE: No problem.

THE COURT: All right.

MR. KOCHIS: Dr. Sensabaugh we would call as our first witness.

THE COURT: Come forward, sir, please.

G E O R G E F. S E N S A B A U G H, called as a witness by and on behalf of the People, was duly sworn, examined and testified as follows:

THE CLERK: You do solemnly swear that the testimony you are about to give in this action now pending before the Court shall be the truth, the whole truth and nothing but the truth, so help you God?

THE WITNESS: I do.

THE CLERK: State your full name and spell your last name, please.

THE WITNESS: George F. Sensabaugh, S-e-n-s-a-b-a-u-g-h.

MR. KOCHIS: May I proceed, your Honor?

THE COURT: Yes, sir.

///

///

DIRECT EXAMINATION

BY MR. KOCHIS:

Q Dr. Sensabaugh, what is your current occupation or profession?

A I'm an associate professor of forensic science at the University of California at Berkeley.

Q How long have you been so employed?

A I've been at the University of California since 1972 on the faculty.

Q And have you had any educational background that qualifies you for that position?

A Yes.

Q What does that consist of?

A I received my doctor of criminology degree from Berkeley in 1969. My area of work was forensic science.

Q What type of classes do you currently teach at the University?

A I teach several classes. The most pertinent in this particular case presumably would be an advanced course in forensic biology, analysis of biological evidence and materials.

I also teach seminar courses dealing with forensic science in general. I teach an undergraduate course which deals with basic human biochemical genetics and a graduate course dealing with methods and microbiology.

Q Between the time you received your doctor in criminology

1-5

in 1969 and today's date, did you have any research or professional experience in this field?

A I had research experience. It wasn't forensic science per se. I did a two-year post-doctoral in the chemistry department at the University of California at San Diego, and I did another year in research at the National Institute for Medical Research in London, England.

Q Have you written any articles which have been received for publication that dealt with the area of serology or criminalistics?

A Yes.

Q A number of those articles?

A Yes.

Q Directing your attention to what has been marked for identification as Exhibit 7, do you recognize what this appears to be a copy of?

A Yes. It's my curriculum vitae.

Q And in that document, are there listed the approximately 90 articles that you have written which have been accepted for publication in some type of scientific journal?

A You are looking at the presentations that's 90. There are 32 articles that have been published in journals. There have been some 90 presentations before scientific societies or scientific meetings.

Q Does that vitae also reflect the professional societies

1-6

that you belong to?

A Yes, it does.

Q Are you currently on any editorial board of any forensic societies?

A Yes. I'm on the editorial board of the Journal of Forensic Sciences which is the journal of the American Academy of Forensic Sciences.

I'm also associate editor of the Journal of Forensic Society, which is an English organization.

I have recently been placed on the editorial board of the American Journal of Forensic Medicine and Pathology.

Q What do your duties on those editorial boards consist of?

A Principally of refereeing articles. When papers are submitted for publication, they are reviewed by two or more referees who review the papers for the suitability for publication. The members of the editorial boards of the journals are the primary referees for articles submitted to those journals.

Q Doctor, does the concept of serology have any application in the forensic field?

A Yes, certainly in the analysis of blood evidence, semen evidence, evidence of a physiological nature.

Q And have you testified in the past as an expert in courts in this state on the area of serology?

A Yes.

Q Within your scientific community, within the community

1-7

of forensic criminalists, is there a concept which is recognized called the ABO blood group system?

A Yes.

Q And is that concept recognized in sciences other than the science of criminology?

A Oh, certainly.

Q And does that essentially hold, that particular concept, that everybody has a particular type of ABO blood group in the blood in their body?

A Yes.

Q Are there essentially four main blood types within that system?

A Yes.

Q What would those be?

A A, B, O and AB.

Q And is it accepted within your community that these various blood types are inherited genetically?

A Yes.

Q And furthermore, they remain constant throughout the lifetime of a particular person?

A Yes.

Q Likewise, are there methods within your community that are accepted as valid and reliable means of typing, for example, a sample of whole blood, to determine what the ABO blood type is?

A Yes. And not just within our community. It's one of

1-8

the basic foundations of blood banking.

Q Within your community, is there any particular definition attached to the two terms I have previously used, those being validity and reliability?

A Validity, you said?

Q Validity and reliability. For example, does the term "reliability" have any meaning within the scientific context?

A Reliable means that you can repeat something and get the same answer.

Q And validity would mean what within the scientific context?

A That the concept has some reality, the same as it would mean in any other context.

Q Returning to the whole blood sample for a moment, is the agglutination technique a technique that's accepted in your community as a valid and reliable means to analyze a sample of whole blood to determine the ABO blood type of that blood?

A Yes, it's one of the standard procedures. It's also one of the standard procedures in basic genetic studies where blood typing is done.

Q Within your community, Doctor, when one is referring to blood which is dry, is the term used "bloodstain"?

A Yes.

Q Are there, within your community, methods accepted as

1-9

a valid, reliable means for analyzing a bloodstain to determine the ABO content of that stain?

A Yes.

Q Would two of those be absorption-elution and lattes test?

A Yes.

Q Is it possible for you to briefly describe what the absorption-elution tests consist of?

A Yes. The absorption-elution test works as follows.

If you have a bloodstain or a piece of blood on a fabric or something of that sort and you add to that a solution containing antibody, antibody against the appropriate blood type, if the bloodstain is of the same type as the antibody, then the antibody will be absorbed onto the blood stain glass, the absorption step, then the stain is washed very thoroughly to remove any known specifically binding antibody. Then the stain which has antibody is heated and the heating releases the bound antibody and you do a agglutination test to determine whether you have released antibody.

If the blood stain is of the appropriate type -- that is, if the blood stain contains that ABO type that the antibody recognizes -- then you will get a positive test.

If, on the other hand, the blood stain is of a different type that does not have the ABO blood group

1-10

antigen that the antibody recognizes, then there will be no antibody found and, accordingly, no antibody released in the elution step to be detected.

Q Is it likewise possible for you to articulate briefly the method employed in the last one?

A Yes. People who are blood group O, for example, possess in their blood plasma antibodies against, that recognize A antigens and B antigens. People who are blood group B contain antibodies against A antigens. People who are blood group A have antibodies against B. People who are blood group AB do not have any of these antibodies.

The Lattes test attests the presence of those antibodies. The stain is solubilized and then the stain extract is tested for the presence of agglutinating antibody.

Q Are these both tests that you yourself performed in the past on blood stains to determine the ABO blood type?

A Yes.

Q Is it possible for you to estimate approximately how many times you have participated in such a test?

A I'm not sure that I would want to hazard a guess. Most of my use of these is in the form of teaching people how to do it. It's done in a classroom context and some number of times per year over the last ten years or so.

Q And are both of these methods accepted as valid,

1-11

reliable means within your community to type a bloodstain for its ABO type?

A Yes.

Q Likewise, is there a concept which is accepted in your community that relates to serology of a person being either a secretor or nonsecretor?

A Yes.

(No omissions.)

Q And the definition of a person who is a secretor would be what?

A A person who is a secretor has soluble ABO blood group substances in their secretions.

Q And would an example of such a secretion be, for example, saliva and semen?

A Yes. Thus, if a person is blood Group A and they are a secretor, they will have blood Group A substances present in their saliva and semen.

Q And then would a non-secretor be a person who either does not secrete those antigens in their blood fluids or secretes them to a much less extent?

A The basic definition of a secretor is someone who does not secrete. And for saliva, that applies. There have been some suggestions that people who are non-secretors may secrete very low levels of ABO substance in their semen, but those are in some contention.

Q Likewise, is there what is recognized in your community a concept called the Lewis blood group system or Lewis type?

A Yes.

Q Is their any relationship that's accepted within your community to exist between a certain type of Lewis blood group person and whether or not that person is going to be an ABO secretor or non-secretor?

A Yes.

Q What is the relationship?
A If a person is a non-secretor, if you test the red cells for Lewis, they will be Lewis a. If they are a secretor, they will be Lewis b.
Q Likewise, is it possible to test whole blood looking for the Lewis types to make a determination as to whether or not a person is a secretor or non-secretor?
A Yes. You do this by testing the red cells for Lewis a and b antigens.
Q Is the microcapillary tube test a test that is recognized in your community as a valid and reliable means to analyze a whole blood sample to determine the Lewis type to in turn determine whether or not someone is a secretor?
A Yes, it's one of several.
Q Likewise, are there tests that can be performed on the other body fluids -- for example, a semen stain or a saliva stain -- to determine if the person who deposited that stain is a secretor or not?
A There are, but it is a more indirect route of testing.
Q Well, starting, first, let's start first with semen. Is there a test that can be employed on a semen stain to reach a determination as to whether or not the person who deposited that stain is an ABO secretor or non-secretor?
A If you test the stain and you find ABO blood group substances, then you know that the person is a secretor.

Q What type of test could be performed on someone who is an ABO secretor to give you that result?
A That would be agglutination-inhibition test. You would be testing for the presence of the soluble substances in the semen.
Q And if you perform that test on the semen stain from a non-secretor, would you then have to perform an additional test or could you just do the absorption agglutination-inhibition?
A It would be wise to do an additional test. If you've got a negative test, then there are two possible explanations. One explanation is that the semen sample originated from a non-secretor and there were no substances there to begin with. The second possibility is that there were substances there to begin with, but for one reason or another those substances have become undetectable. So one really in order to say that this -- a particular sample came from a non-secretor, one would have to distinguish between those latter two alternatives.
Q Would one such additional test that could be performed be the absorption-elution test?
A Yes. The absorption-elution test would detect antigen on the cellular material in the stain, the sperm and other cellular debris. And if you found antigen on the cellular material but failed to find antigen in the

soluble part of the stain, then that would be indicative of a non-secretor.

Q Could you likewise test for the Lewis system on semen stain to get an indication as to whether or not a person was a secretor?

A You can. There are several published papers on the use of Lewis in that regard. The amount of material in the stain would have to be reasonably concentrated, because the amount of Lewis substances in the stain, in semen, is not nearly as high as the amount of ABO substances in semen.

Q Could you describe briefly for us the agglutination technique you would perform on a semen stain to determine whether or not initially someone was a secretor?

A You mean the agglutination-inhibition?

Q Yes.

A Yes. The basic principle of this test is to mix specific agglutinating antibody at a low titer so that there is just enough antibody present to agglutinate the red cells, to mix some of this antibody with an extract from the stain material. If there is soluble substance present in the stain, the soluble antigens will react with the antibody and effectively neutralize the antibody. Then when after some period of time you add indicator red cells of an appropriate type, if the antibody had been neutralized -- that is, if it is reacted with

soluble substance -- then there will be no agglutination of the red cells.

If on the other hand soluble substance is absent or present at very low levels, there will remain agglutinating antibody and the agglutinating antibody will agglutinate the indicator red cells.

Q Doctor, directing your attention again to a stain, is there or are there accepted methods within your field to test a stain to determine at least presumptively whether that stain is a semen stain or stain from some other body fluid?

A Yes.

Q For example, is one of the presumptive tests that is recognized in your community the test for acid phosphatase in the stain itself?

A Yes.

Q And is there a name that is used in your community for that particular type of test?

A The acid phosphatase test.

Q Can you also do a follow-up check on that test by looking at the stain underneath a microscope to determine the presence of sperm?

A Yes, indeed. That is the definitive test for semen, since sperm is found in no other body fluid.

Q With saliva, are there presumptive tests that are used on a stain to determine whether or not it may be in the

form of saliva or from some other body fluid or other substance?

A Yes. The test there is for an enzyme that is present in saliva at very high levels. The name of the enzyme is amylase.

Q If you find amylase in a stain, is that conclusive proof that it is in fact saliva or is that simply a presumptive test?

A No. That would be presumptive, although the amount of amylase that one finds would enter into the weight of one's presumption. The amount of amylase in saliva is incredibly high, and if one found incredibly high levels, then that would be taken as very strong evidence of saliva. If one found moderate levels, that would be less weighty evidence for the presence of saliva. This is also predicated on a knowledge of other fluids in which amylase appears at any reasonable level.

Q Doctor, if a stain is analyzed, suspected saliva stain is analyzed, and, let's say, a high concentration of amylase is found -- and I'm trying to recall if I mis-spoke myself.

If a saliva stain is analyzed and you find high concentration of amylase, are there then tests you can perform on the stain to determine if a person is a secretor or non-secretor?

A One would test by the agglutination-inhibition in exactly

the same way. If one finds the presence or if one finds secreted substances present in the stain extract, then the person is a secretor. If on the other hand secreted substances are not found, then the person is either a non-secretor or there were secreted substances there and they for one reason or another are not detectable.

Q Would then you have to perform a follow-up test?

A One could actually do the same kind of thing that one can also do for semen, which I did not mention earlier. I'll explain for saliva first.

If one found such high levels of amylase present in a stain, then knowing how much blood group substance is present in saliva, and we do know, have an idea how much blood group substance is present in saliva, one could infer that this could not be a saliva stain that contained blood group substance at an undetectable level. One can do the same thing with semen stain, in fact, that if by testing the acid phosphatase activity, if we find very high levels of acid phosphatase, because we know how much blood group substance is ordinarily present in semen, we can distinguish between the possibility of a low level, low to undetectable level blood group substance, we can distinguish that possibility from there having been no blood group substance there to begin with.

Did that come across clearly?

Q Clearly is a relative concept. I don't know how to

answer that.

Is it accepted within your community that these various ABO blood types occur with a certain frequency within a given population?

A Yes.

Q In fact, have studies been done to bear that out?

A Oh, yes, yes. There are extensive studies on the distribution of ABO blood types in various populations.

Q Likewise, have studies been done as to the occurrence of secretors versus non-secretors in a given population?

A Yes, somewhat less extensive, but some studies have been done.

Q And the techniques that you have described earlier, for example, to determine the ABO type of whole blood and blood stains, is that something that has been in use in your community only recently or for some period of time?

A The ABO type in stains, you said?

Q Yes.

A It's been in use for at least 20 years.

Q Are there genetic markers other than the ABO system which manifest themselves in the blood system?

A Yes.

Q And is an example of those enzymes and serum proteins?

A Yes.

Q Is it accepted within your community, within your scientific community, that there are various enzyme types

and serum protein types which exist, for example, in the blood of all of us?

A That is correct.

Q Is it further accepted in your community that within a specific enzyme type -- for example, PGM or EsD -- that there are different PGM and EsD enzyme types?

A Yes.

Q And have there been studies about tests that are used to determine the presence of a particular enzyme in a bloodstain?

A You mean the determination of a particular type?

Q Particular type.

A Yes.

Q Is electrophoresis such a test?

A It's the principal tool that is used for the analysis of the enzyme and protein genetic markers.

Q And basically what does the electrophoretic test consist of?

A The genetic markers are characterized by specific charge states. Two different types of a given enzyme will have a different electric charge, so if one places the protein in an electric field, the proteins will move in the electric field at a rate that corresponds to the charge they have, on the electric charge they have on them. And because two types -- if two types have different charges, they will move to different positions in the

electric field. And so one recognizes the types by the pattern that is obtained by the marker in the electric field.

(No omissions.)

3-1

Q In the laboratory, does that take place by using typically a glass plate, for example?

A The electrophoresis is done on some medium which may be supported on a glass plate or on a sheet of plastic.

Q And is the medium typically some type of gel?

A It's usually a gel, although other media can be used effectively.

Q Are standards and stains then placed in the gel which is actually on top of the glass plate?

A That's correct.

Q And then is an electric current run through the gel?

A That's correct. You take your extract and your standards and you pick them up on pieces of filter paper or on threads and either lay them on top of the gel or insert them in slots on the gel. Then you apply the electric field and the markers migrate off of the threads or off of the filter paper through the gel at some rate, and then, at the end of the electrophoretic run which has been the type which is determined empirically, you turn off the current and then stain the gel to determine the location of the particular marker in which you are interested.

Q Now, Doctor, approximately how many different types of enzymes and serum proteins have been found in the blood, for example?

A Well, over 50 and approaching a hundred, I would guess.

3-2

I have a table of them somewhere, but I haven't counted them. I don't remember the exact number in the table. It runs three pages long and that's what I do remember.

Q Is every enzyme which has been discovered used in your community to type or distinguish blood stains?

A No. No. Of the genetic markers that are known, which I said, the number is somewhere between 50 and a hundred, perhaps only a dozen to 20 have proven sufficiently robust to be used in the forensic context.

What I mean by robust is that the marker has to survive in the stain material in a form which allows it to be typed without ambiguity.

Q Now, how, in your particular field, how do you use forensic serology to go about determining which particular enzyme is the suitable field to use in? Do you do it by chance or alphabetical order or test it?

A No. Usually it starts off when someone publishes a paper saying that a new genetic marker has been found, so the people in the Crime Laboratory or people in some crime laboratories will read the paper, they will buy the appropriate reagents to do the test, they will try it out. And if they can get the marker to type cleanly off of fresh blood samples, then they will prepare some sample stains and they will see whether the marker survives in the stains.

Some markers, when you prepare stains, the marker

3-3

simply will lose activity. Some markers, when you put them into stains, become -- they give unclear patterns, ambiguous patterns. Those markers would be rejected. The markers that give clear typing results would be accepted for further work.

The second stage would be, then, the preparation of a number of stains, storing them under different conditions for different periods of time, testing them in a blind way. And if, at the end of that testing, mistakes have not been made -- that is, somebody knows what the types are but the analyst who does the analysis does not know what the types are -- if the analyst, in making his or her typing calls, is correct all the time, then the test and the marker are considered sufficiently reliable to introduce into case work.

Q So for us laymen, in this two-step process, the first step is essentially some sort of test to determine if a particular enzyme type will manifest itself clearly?

A Yes. Feasibility study. Find out whether it's feasible, whether this is a feasible marker to work with.

The second stage would be the reliability trials.

Q Would that consist of exposing various stains, for example, to differences maybe in age, heat, allowing time to pass and then having people who aren't aware of that genetic makeup analyze the stain?

A The people who don't know what the answers are analyze

3-4

the stain and then they see whether the answers they come up with are the correct answers.

It's just like taking a test. When you take a test, you don't know, if you haven't studied, you don't know what the answers are. If you have studied, you put down the answers you think are correct.

There is one who knows what the answers are who sits in judgment and decides whether or not you have given the correct answers.

And so basically, the trial testing is that kind, that blind testing is that kind of process.

Q From this two-step process, you whittle down the number of known enzymes into those that can be reliably typed?

A That's correct.

Q Directing your attention to one of the charts which is behind you on the board, directly behind you, and for this hearing, I had marked for identification as K-1, are you able to read, for example, on the line that says "Group I" and it says, I believe, PGM and and EsD, are you able to read that portion of the chart?

A Yes.

Q Now, are the items EsD and PGM, are they examples of two types of enzymes that have passed this two-prong test within your community?

A Yes.

Q Are they enzymes that your community recognizes as being

3-5

typed by this procedure called electrophoresis?

A Yes.

Q Would an agarose/starch gel be an acceptable medium to use in an electrophoretic analysis to determine these types of enzymes?

A Yes. That's one of several that have been used successfully and reliably.

Q Directing your attention to the chart, the buffer that's listed on that particular chart, would that be an acceptable buffer, at least recognized in your community as an acceptable buffer to use in electrophoretic run to determine the enzyme types?

A Yes. Basically, that was one of the original buffers developed by human geneticists to determine phosphoglucomutase.

Q Likewise, would 7.4 be an acceptable pH with which to conduct that electrophoretic run?

A Yes. Once again, that was the standard's original condition.

Q Likewise, finally, the stains that appear on the chart to the far left of the Group I under "staining", would that be appropriate stains to use in conducting an electrophoretic run to determine PGM and EsD?

A Yes, those are the stains used by everyone who types for these particular enzymes.

Q Are there any controls that are used in the

3-6

electrophoretic run to allow an analyst to determine if the system is functioning properly?

A Yes. You run standards, samples of known type.

Q How would that function for a serologist as a control?

A Well, if the known type does not give you a good pattern, a prototypic pattern, then you know that something was wrong, went wrong somewhere in the analytical system. It doesn't happen very often, but it occasionally happens.

Q When you are talking about patterns, would the pattern of the unknown itself also operate in any fashion as a control?

A If the pattern -- Yes, if the pattern of the unknown deviates significantly from the kinds of patterns that one expects to see, then that would serve as a control.

There are, when you do these, use these typing methods, there are expected patterns that one expects to see. You are one type, you manifest one pattern, or you are another type, you manifest another pattern.

If the pattern that shows up on the plate is different than any of the expected patterns, then an alarm bell rings that something is not right.

Q Doctor, when you are talking about patterns, I wonder if I could direct your attention to another exhibit which is behind you on the board. And I believe that one has been marked for identification as K-3.

3-7

Can you read that?

A Yes.

Q Is it K-3?

A Yes.

Q For example, the diagram of the different types of PGM, the standard PGM types, can you see those?

A Yes.

Q And, for example, if you use a standard in electrophoretic run, a 2-1 standard, and you have also had a sample of blood that was not known to you and you got expressions similar to the 2-1 on that diagram, would that first be an indication from the control that the run had been conducted properly?

A Yes.

Q And likewise, if your unknown blood manifested a similar pattern, would that also operate as a similar control that the run had been conducted properly?

A If your unknown blood gave a 2-1 pattern?

Q Right.

A Yes.

Q Now, I take it there are things that can affect the electrophoretic run, for example heat and voltage. Would that be true?

A Yes. If the voltage is too high, a great deal of heat may be generated. If the heat is not sufficiently removed, then you may get heating of the gel which would

3-8

lead at its minimum level to no serious problem to at a more distortion of the bands to the most extreme, the gel would burn up.

Q If there was a heat problem such as the one you just described, would you expect that problem to manifest itself on the standards?

A Yes.

Q How so?

A If the standard showed up giving a very blurry pattern or if the pattern given on the gel was not uniform across the gel but smeared or skewed to one side or another, that would be an indication of a heat problem.

Q Doctor, directing your attention back to the chart directly behind you, the K-1, are the three enzymes that appear adjacent to the Group II, the EAP, ADA and AK, are those all examples of enzymes which have met this two-prong criteria about validity in terms of being able to type from the bloodstain?

A Yes.

Q Is that accepted by serologists other than yourself within your community?

A Yes.

Q Is the use of a starch gel accepted in your community as a valid and reliable means to conduct an electrophoretic run on those three enzyme types to determine their type?

3-9

A Yes.

Q And likewise, the other information that appears on the chart next to Group II, specifically the buffer that's listed on the chart, the pH that's used, and the staining, types of stains that are used, do those all appear to be appropriate procedures to employ?

A Yes. They are fairly standard. These procedures are, for the most part, no different than procedures that are used by a biochemical geneticist who is doing nonforensic work.

Q With electrophoresis I assume that it's proper to conduct a run to determine simply a single enzyme type, for example PGM; is that true?

A What do you mean by a single enzyme type?

Q Is it proper to analyze a bloodstain on a plate in an electrophoretic run just searching for the PGM enzyme type?

A It depends upon the context. If the PGM is the only enzyme you are interested in, yes, that's appropriate.

On the other hand, if you are interested in getting a general picture of the genetic types of the individual, then you may want to stain a single gel for other enzymes as well, if you know the other enzymes will work.

In the process of doing population genetic studies, the electrophoretic run is a rather limiting process. You can only analyze as many samples as you can get

3-10

through your electrophoretic analysis. The staining step doesn't take much time. The electrophoresis step takes a long time.

Very early on in genetic studies, people found buffer systems that would work well for several different enzyme markers, so one could then run a fairly thick gel and slice the gel into a number of slices and stain each slice for a different enzyme and that way you could get as many as five or six markers off of a single electrophoretic run.

Q I think you may have answered the next question I was going to ask but I take it within your community it's accepted as valid and reliable to use a multisystem and look for more than one enzyme type?

A Yes.

Q And are you aware of a person by the name of Brian Wraxall?

A Yes.

Q And has he designed what is called the multisystem which is employed in the country ~~in various crime laboratories~~ to run more than one enzyme type off the same one?

A Yes.

Q Likewise, directing your attention again to K-3 -- sorry -- K-1, are transferrin and Gc examples of proteins that have met the two-step criteria as far as reliability?

A Yes.

3-11

Q Is an agarose gel accepted as a reliable means of conducting electrophoretic runs to determine the Gc protein?

A It's the standard procedure that is used, yes, or one of the standard procedures.

Q Likewise, on K-1, moving along the line of Group III, are the buffers, the pH and the staining technique that appears on the chart proper procedures to employ in conducting a run for those serum proteins?

A Yes. Those are fairly routine procedures.

(No omissions)

Q And are peptidase A and CA II also examples of enzymes which are accepted in your community as enzymes which have met this two-stage process and they in fact can be reliably typed?

A Yes.

Q Is electrophoresis recognized in your community as the method to make that determination?

A Yes.

Q On the chart, the gel that's reflected there, the agarose as well as the buffer, the pH and the staining, are those acceptable procedures to employ in an electrophoretic run where you're looking for peptidase A and CA II enzymes?

A Yes.

Q Also, is haptoglobin an enzyme that your community recognizes can be reliably typed?

A Yes.

Q Would an acrylamide gel in electrophoresis be accepted, is it accepted in your community as a valid and reliable means to make that determination?

A Yes.

Q And on the chart are the buffers, the pH and the staining technique that is used, accepted as the proper method to conduct that type of run?

A Yes.

Q Now, are there changes that can occur in blood -- perhaps

I can use the word wet stain and dry stain changes -- can they affect the ability of the serologist to later type using electrophoresis the enzyme types of that particular blood?

A Could you rephrase that, please? I think what you're trying to say -- correct me if I'm wrong -- is are there things that can happen in bloodstains that cause bloodstains to become untypable?

Q That's a better question. What would your answer be if I asked it?

A There are.

Q For example, can bacteria affect a bloodstain?

A Bacteria do, yes.

Q If bacteria affected a bloodstain, would you expect the presence of the bacteria to manifest itself in an electrophoretic run?

A It might manifest itself several ways. If I may have the latitude to answer the question broadly, since now we're moving into lecture territory, to have bacterial overgrowth of a bloodstain requires that the bloodstain be maintained in the liquid state for some period of time.

Q I was going to stop you just for a minute. For example, as a forensic serologist, are there steps you can take to arrest the possibility of this occurring?

A Drying it and refrigeration. We dry food to keep it, to protect it against bacterial growth. We freeze our food

to protect it against bacterial contamination.

Q Likewise, if a drop of blood is deposited, for example, on a surface such as a wall and kept away from moisture and later collected in a dry form and frozen, would that lessen the chance of any bacterial contamination at that state?

A Well, during the time that the stain is in the dry state and frozen, bacteria will not be a problem. Any problem that might occur would be during the time that the stain is in the liquid state on the wall. We have some idea of how long it takes blood spots to dry under normal conditions. And so we could come up with a reasonable estimate of the amount of time the blood was in the liquid state. And with some outer limits as to what was possible and within that time we could make a rough estimate of how much of an effect bacterial contamination might be.

In any case, if there was significant bacterial contamination, you would see it when you were looking at the stain. Really significant bacterial contamination would show up in the form of a discolored stain; for example, stain that has mold on it, and we've all seen moldy food in our refrigerator. Well, moldy bloodstains look about the same way. You know, when you have a contamination problem of that sort it's painfully apparent.

If you don't have that level of bacterial contamination or fungal contamination, then chances are it won't have an effect on your stain, on the analysis of your stain material. In other words, you have to have a pretty fair amount of contamination in order to have a significant effect on the analysis on the markers in the stain.

Q If I could stop you for a moment so I can digest some of that.

Do you mean to say by some of that two things, doctor: First of all, the existence of contamination in and of itself may not affect the markers, in terms of their manifestations; and second, if bacteria does affect the stain, for example, adversely, that's going to manifest itself in the electrophoretic run?

A Well, it will usually manifest itself just looking at the stain visually. Now, when you do the analyses, the electrophoretic analyses, and for that matter the ABO typing analyses, one, particularly the electrophoretic analyses, if you have bacterial contamination in a stain that is significant enough to affect the stain, you will usually see some bacterial representatives or bacterial forms and enzymes present.

So, for example, with PGM, with bacterial contamination, stains that have been subjected to or have undergone bacterial, significant bacterial contamination, one often

sees a PGM band that is far removed from the bands that one does the typing on. If you see a band like that, that's a sure signal that you have a problem.

Q Doctor, I wonder if you could move to the blank piece of paper that we'll later have marked, I guess, as K-8 for identification. And would it be possible for you to demonstrate, for example, with PGM on the left, perhaps, what a normal PGM_1 type would look like and then what a PGM_1 type that has been subject to contamination by way of bacteria might appear?

A Well, the ones that have been looked at thus far, if we put the mark of origin here, and using those same patterns, a PGM Type 1 is represented by two bands. There are another series of bands which are found in all individuals and which do not show genetic variation. These non-variable bands are due to the PGM_2 locus. It's a separate enzyme system, but it has the same enzyme activity. And then when bacterial bands have been seen, they tend to be up here.

THE COURT: Go to the other side if you will, please.

THE WITNESS: I'm sorry.

Q (BY MR. KOCHIS:) Could you draw a line between the bands that appear in a normal stain and the bands that appear in a bacterial stain?

A Anywhere in there.

Q And that would obviously manifest itself in the plate

during an electrophoretic run?

A Yes. Now, this doesn't mean this would have to be where those bands would show up. This is where the bands, the contamination bands that I have seen, have shown up. They could show up in other places on the plate as well.

But the point is that as soon as you start seeing funny bands, that is, bands in places where bands should not be, that is a signal that something is not quite right.

Q Now, have you written literature about the various things that can occur that will affect electrophoretic analysis of blood?

A Some, yes.

Q For example, did you essentially author a chapter in, I believe, Saferstein's handbook?

A Yes.

Q And was that essentially to be used by serologists as listing the various signposts that they are to look for when they analyze a particular stain to determine if the stain has been affected by a wet or a dry state change?

A Well, it describes the basic biochemistry of some of the marker or outlines the basic biochemistry of some of the markers and describes some of the kinds of things that one can see when things are going wrong, yes.

Q Now, does the fact that dry or wet state changes may occur, does that mean in any fashion that bloodstains cannot be

reliably typed to determine their enzyme type?

A No, because the consequence of the changes that do occur, as we have gone through here for the bacterial contamination, the consequences are that the patterns are sufficiently altered to signal that something is not right. And if the patterns are altered, then one relies on one's knowledge of the marker itself, the biochemistry of the marker, one's experience as to whether one is actually going to make a call, or whether one will withdraw, be agnostic, and say I don't want to commit on that particular pattern.

Q Doctor, do enzymes lose their activity with time?

A Yes.

Q And when an enzyme loses its activity, will it eventually discontinue to express itself?

A The pattern disappears.

Q And if an enzyme, for example, these various enzyme types we've discussed, degrades to that point, is what practically happens that you simply won't get anything on the plate?

A That's right. The markers that we know have two basic patterns of decay. One basic pattern of decay is the activity just disappears, it fades away. And it fades away evenly, so that one type does not fade away more rapidly that another type.

The second pattern of decay is that we have

degeneration of additional electrophoretic bands. We know where those electrophoretic bands ought to go. The patterns are rather well defined patterns. And at some point in the decay process, the pattern becomes sufficiently altered that the analyst says I don't want to make a call.

Q So that type of change also manifests itself on the plate?

A That's right.

Q Now, is it also possible to conduct an electrophoretic analysis of stains other than blood to determine enzyme type; specifically here I'm referring to semen?

A Well, PGM and peptidase A being two principal semen markers.

Q Is it recognized in your community that both those enzymes can be reliably typed through the use of electrophoresis to determine, for example, the PGM and peptidase A enzyme type of a particular semen stain?

A Yes.

MR. KOCHIS: I have nothing further on direct.

THE COURT: Why don't we take the morning recess, about 15 minutes. We'll be in recess.

(Recess.)

(No omissions.)

5-1

THE COURT: Doctor, return to the stand, please. You are still under oath to tell the truth.

Mr. Negus, you may cross-examine.

CROSS-EXAMINATION

BY MR. NEGUS:

Q In determining whether or not these different enzymes that you have been talking about are accepted in the scientific community as reliable, does publication play any role in that?

A Yes.

Q What role does that play?

A Well, when someone has gone through the kind of testing that I described, they would generally publish their results. Either that or they will present their results at a meeting. And from one or the other, they will pass the words to the rest of the community that they feel that these tests are sufficiently reliable to be used in case work.

Q Generally, does the process of getting the word around by publication take some time after initial discovery?

A Yes.

Q Is there ever occasions where somebody thinks something is reliable and publishes it and then later they find out that it wasn't?

A Yes, that can occur.

5-2

Q For example, at one point in time immunoelectrophoresis process or technique was thought to be reliable; is that correct?

A It was published and I wouldn't really say it subsequently proved to be unreliable so much as there are other techniques that give more definitive results or are easier to interpret and have supplanted the immunoelectrophoresis technique.

Q This is unreliable?

A I don't really harbor any such suspicion. I wouldn't use it because I think it's a pain to do, and the number of no-calls, I would judge, would be considerably higher than the number of no-calls using the acrylamide gel techniques that are used now.

Q Generally, how wide a circle of serologists generally hear about these things before they are accepted as reliable? I mean, just those in California or all over the world?

A Well, I'm not sure exactly what you are getting at, but there is a worldwide community. There are groups in Japan that do work on the markers. There are groups in Western Europe, groups in England and in this country. There are several laboratories in each one of those places that have tended to be the ones who do most of the testing work, and they will present their papers either through publication or at appropriate meetings

5-3

and pass the word along.

Q For example, three of the main ones would be London, California and Tokyo; is that correct?

A I'm not really very familiar with the Japanese work. Certainly work has been done, a substantial amount of work has been done in London. There has been some work by several groups in Germany, the location of which I cannot define for you because they publish in German. I read the abstracts, but not the papers.

Q Well, the PGM, the different techniques for PGM have been widely distributed throughout all the different countries; is that correct?

A Yes.

Q And how many of the particular proteins that are on that list behind you have been the subject of published articles describing the technique that is on the board in the various scientific journals?

A You mean how many publications have used these particular, or how many publications have these particular techniques been used in?

Q How many of the enzymes have been described in any scientific publication with the techniques that are described on the board?

A I would have to go back. To answer that definitively, I would have to go back and look at the specific articles and see what techniques have been used. The techniques

5-4

that have been used or most of the publications regarding phosphoglucomutase have used the same buffer system, same pH. They have been done, for the most part, on stain, not on starch areas.

There have been some in which agarose has been used by itself. With regard to the acid phosphotase, that is one of the fairly standard procedures that has been used quite a lot in biochemical genetics -- that is, using the citrate phosphate system at that particular pH or at a pH 5-8 or 5-9. It has a little bit of difference in the way the patterns appear, but not a significant difference.

Q Have there been publications in scientific literature describing the use of agarose gel to get transferrin results in a forensic context?

A In the forensic context, I'm not sure there has been. In the basic genetic context, I know there have been papers presented in that regard. I know the people who have presented the papers haven't published that work.

Q Basically, the general description of the technique used to group transferrin in Group Specific Component together were presented in 1979, a paper by Ed Blake to the California Association of Criminalists?

A The name is right and the date sounds about right.

Q Then he also published, also presented in 1983 a couple of papers on certain problems that could develop using

5-5

transferrin?

A Yes.

Q Those again were in the California Association of Criminalists?

A Right. And then they also have been presented at one other forum, a forensic science meeting, the name of which I don't remember.

Then I presented them for Ed at a Canadian meeting a year ago last August or last August. I think it was last August.

Q Is it fair to say that a technique has been generally accepted in the forensic science community if its only presentation has been in groups, to certain groups at certain meetings and hasn't been published in a scientific journal?

A That has both a yes and no answer to it. If I may elaborate, if the technique that is presented poses nothing particularly novel -- that is, it's a modification of an existing technique or existing technology -- then that would be considered support for the reliability of it.

On the other hand, if the technique were entirely a novel technique, something that had no precedent, had not been done before in that particular way, it would not be considered really established as reliable; rather, it would be considered as provocative and

5-6

something that other labs would want to take back and try out for themselves.

Q In the reliability of these various techniques, is it necessary that an analyst be generally familiar with this literature in the field?

A Yes, and basically with the biochemical genetics literature as well.

Q For example, you mentioned to Mr. Kochis a review article that you presented for Mr. Saferstein in the Handbook of Forensic Sciences.

Would an analyst need to know at least the material summarized in that article in order to be able to reliably make a typing?

A I should think that an analyst ought to be familiar with that material. That was the rationale for bringing it together in that review article.

Q Could ignorance of that material cause unreliability?

A I'm not sure exactly how to answer that question because there you are getting not at the question of reliability of the marker or the reliability of the method, but, rather, the competency of the analyst. And if the analyst is not familiar with that material, then I would say that the analyst might have a greater chance of making a mistake than the analyst that was familiar with the material.

Q Some techniques are less acceptable to analyst

5-7

misinterpretation than others in the field of forensic science; is that correct?

A As in any field, yes.

Q The gaschromatograph, when you analyze with it, that's reasonably free of misinterpretation; is that correct?

A It ought to be, yes.

Q Whereas something like neutron activation analysis, at the other end, takes a considerable amount of skill just to even operate the machine, correct?

A Yes. It is really at two levels. One, the technical skill to prepare the samples and operate the machine, and, secondly, to interpret what the results mean.

Q Between the gaschromatograph for ethanol and activation analysis, where does the electrophoresis fall?

A Somewhere in the middle. The technology of doing the electrophoresis is not particularly difficult. Indeed, in most chemical laboratories where the electrophoresis is done, or blood banking laboratories where it's done, it's not uncommonly done by technicians who have no particular training in any fundamental biochemistry or genetics, but they do know how to prepare samples in a standard way, how to put the samples onto a gel, how to plug in the machine and do the analysis.

So at the technical level, there is not necessarily any great expert involved in interpreting what the patterns mean, if the patterns are unambiguous.

5-8

Generally, there is no particular special expertise that's needed. It's when the patterns begin to approach the boundary of the ambiguous that one needs to have the special knowledge.

Q At that level, does one need as much special knowledge to interpret neutron activation analysis?

A Not being an expert in neutron activation analysis, I'm not sure that I could judge. Certainly it would be a different kind of expertise.

Q What kind of expertise? What different things go into the expertise that you need for serology?

A I think you ought to have a basic knowledge of genetics, particularly biochemical genetics, a background that includes serology, some chemistry. I would say the biochemical genetics would be the main thing.

Q And that kind of background would be a sine qua non for interpreting results where the patterns may be a little ambiguous; is that correct?

A Yes. I would say that would be fair to say, yes.

I should add that's not the kind of biochemical genetics that's done at the research level. It would be somewhat more basic or a lower level biochemical genetics than that. We don't expect the people doing this kind of work to be carving away at the frontiers of science, and they don't have to have that kind of knowledge.

5-9

Q And in applying that knowledge, you spoke of the knowledge between the -- of the differences that can occur to these various proteins in a wet environment versus a dry environment?

A Yes.

Q And when we are talking about -- when you are talking about wet and dry, wet can be a, not necessarily immersed in water, but can be present at a certain relative humidity; is that correct?

A Yes.

Q Have you published some work as to approximately where the difference between wet and dry becomes significant in terms of developing humidity?

A Yes. Somewhere between, say, roughly 60 percent and 80 percent of relative humidity. There is no hard bound line between wet and dry in this context, so that 60 to 80 percent is about right.

Q Somewhere between 60 and 80 percent relative humidity the kind of changes that you start seeing the proteins and no longer those characteristics of the dry state but the characteristics of the wet state?

A Above 80 percent, yes.

Q And you published that information in the journal called Isozymes; is that correct?

A It's a serial. It's not a journal. It's a book of review articles that appears every year.

Q What are the different -- well, do all the enzymes undergo the same wet state changes or proteins?

A No, no. It would depend upon the chemistry of the enzymes themselves.

Q By the way, all the enzymes are proteins but not all the proteins are enzymes; is that correct?

A That is correct, yes.

Q Is there a microorganism which is present in the human body called neuraminidase?

A No, it's not a microorganism, but it's an enzyme that is present in the human body. And it can come from microbial contamination. It is also an endogenous enzyme.

Q Is it found inside red blood cells?

A Neuraminidase? Not to my knowledge. Low levels of it may be present in blood plasma, but they would be very low levels. For biological reasons, it would have to be present at very low levels.

Q In people who have died, does the neuraminidase level sometimes increase?

A Yes.

Q Does it at that point in time get inside the red cells?

A No, it wouldn't get into the red cells, but as the red cells would break open it would -- the contents of the red cells might be exposed to the neuraminidase.

Q Does neuraminidase affect any of the red cell enzymes in

producing any sort of changes in it?

A It should not have an effect on the red cell enzymes, because -- and this is a basic biological issue -- the enzymes that are present within the cytoplasma of the red cell, that is, inside the red cell, do not have attached to them sialic acid, which is the substrate of neuraminidase. That is, it is the sialic acid that the neuraminidase acts on. Proteins that are present in the red cell membrane, proteins that are present in the blood plasma, very many of those do have sialic acid on them and they may be affected, may be modified by the action of neuraminidase.

Q Is acid phosphatase, that is acid phosphatase from the red blood cells, is that affected by it?

A No.

Q The three serum proteins that were mentioned to you on Exhibit K-1, transferrin and haptoglobin, both have sialic acid; is that correct?

A Transferrin certainly does, yes. Haptoglobin does, and Gc does also.

Q Gc only has it, however, in some of its phenotypes; is that correct?

A It is more prominent in one phenotype than in another phenotype, yes.

Q So the effect in terms of at least the kinds of typing that we're talking about, neuraminidase, on these various

proteins, is only going to be a significant problem for transferrin, haptoglobin and some of the phenotypes of Gc?

A Well, depends on what you mean by a problem. Actually with Gc, unless you have a rare type, it's not going to be a problem, because the patterns are sufficiently distinct that there should be no confusion there, even if neuraminidase has acted.

The same applies actually for haptoglobin. If the tests are done properly, then the patterns ought to be sufficiently distinct so that you would not make a mistake with regard to haptoglobin type.

With regard to transferrin, if you know what you're looking for or know what to look for, then there shouldn't be a problem there either.

Q Perhaps I shouldn't used the word significant problem. The neuraminidase is only going to affect the electrophoretic appearance of those three proteins?

A Yes, yes.

Q The key to it not being a problem is that you know what you're looking for; is that right?

A Yes.

MR. KOCHIS: Your Honor, do we have any control at all over whatever it is that's making that noise?

THE COURT: I don't know.

DEPUTY SPEARS: I don't know what it is either.

THE COURT: It's something I think we can live with here. I don't know where it's coming from.

MR. NEGUS: I suspect from past experience in this building it's the air conditioner.

THE COURT: It is annoying, but we can overcome it. You might check, bailiff, just to see if you can get more information on it. We'll talk about it later.

Go ahead.

Q (BY MR. NEGUS:) When the blood is in a dried state as in the situation with the blood dropping, for example, that Mr. Kochis mentioned, is neuraminidase going to be a problem for even transferrin, Gc, or haptoglobin?

A Not in the dry state. Enzymes don't work in the dry state. The main effects of bacterial contamination have to do with bacterial enzymes chewing up the material that the bacteria are contaminating. In the dry state the enzymes don't work.

Q Another one of the changes that can occur in the wet state is a process called deamidation; is that correct?

A Yes.

Q Is there a difference between the word deamidation and the word deamination?

A Deamidation, I think, was the term that you are looking for. It is the removal of amide group.

Q What's deamination mean?

A Deamination would be the removal of the amine group.

Q In organic chemistry, amide groups are the various amino acids that makeup the proteins?

A The amine or amide?

Q With a "d."

A Amide. Two amino acids have amide type changes, yes, have amide groups.

Q Is the deamidation a process that's only a problem in the wet state?

A Principally a problem in the wet state. The reaction, the chemical reaction requires water in order to go.

Q So unless, for example, in blood drop, unless that drop was wet at some point in its history for a very significant period of time, deamidation shouldn't be a problem; is that correct?

A Well, that and also again it depends upon the chemistry of the protein or the chemistry of the enzyme. Some enzymes, proteins, have labile amide groups. So it's only going to be if you have a labile amide group that deamidation is likely to be a problem. So it's dictated -- deamination as a problem is dictated by the chemistry of the protein.

Q Now, of those which are the proteins that -- let's just assume on that list that -- well, I'll back up.

Glyoxalase 1 is a protein that can be typed according to Group I; is that correct?

A You can use that typing electrophoretic system, yes.

Q At no extra cost as it were you can get a glyoxalase 1 result at the same time as you're doing the PGM and the EsD?
A Yes.
Q Doing that doesn't affect the reliability of PGM or EsD results if you use that particular system; is that correct?
A If it's done properly, if should not.
Q As far as the Group IV system is concerned, there's another protein called G6PD; is that correct?
A Yes.
Q And that protein can likewise be determined using that system for Group IV at no extra cost?
A It would seem reasonable. That's not the condition that I use for glucose 6 phosphate dehydrogenase. There's no reason why that system could not be used for G6PD.
Q You could likewise using that same system also get hemoglobin if there's some sort of variant in the hemoglobin; is that correct?
A Yes. Actually you can pick up hemoglobin variance on most of those systems.
Q Essentially if it's possible?
A Yes.
Q So adding them, if you would, glyoxalase and the G6PD to that list up there of proteins, which of those in the wet state undergo deamidation changes?

A That's not a question that can be answered, because although we have inferred from the nature of changes that occur that deamidation may be responsible for some of the deterioration phenomena that we see, it has not been proven chemically that deamidation is in fact responsible for it.

Q Well, the phenomena one sees as far as from which one infers a possible deamidation is a shift towards the anode of the bands?

A That is correct, a one-step shift, yes.

Q What's a one-step shift?

A Well, a one-step shift would be like the difference between these two bands in PGM.

Q Between what's been labeled as the little a and the little c band?

A Right, on the Type 1 or little b and the little d band on the Type II.

Q Which enzymes and proteins undergo that particular shift?

A Undergo a one-step type shift?

Q Right.

A PGM does, adenylate kinase does, esterase D does, ADA does for a different reason, peptidase A does, if I remember correctly. I'll qualify peptidase A, because I don't remember that. It's not an enzyme with which I've done extensive work. However, I think if you'll look in the table of the isozymes book, I have a list

which indicates which enzymes undergo a deterioration with formation of anodal bands.

Q In that particular book, you don't mention peptidase A. Is it possible, however, that even because of your lack of work in the field you may not know it and it does?

A I was relying upon published accounts and statements from other people in preparation of that table, so I would guess that it would not. It would be in Volume 11.

Q It's cut out. I'm showing you a cut-out Table 6.

A Right. That would be the table.

Q So the peptidase A would not undergo --

A It would be simple loss of activity, yes.

Q You mentioned that the ADA undergoes this one step and that will change for a different reason. Why is that?

A It forms a mixed disulfide with oxidized glutathione.

Q Is that something that happens only in the wet state?

A With regard to ADA specifically, I can't answer. The chemistry of it says yes, because the same thing occurs with red cell acid phosphatase. And as far as we have been able to do tests, the formation of the anodal bands with oxidized glutathione occurs in wet state material, but not on dry state material.

Q Basically in order to get these changes, especially, it adds a negative charge that makes it go further?

A Yes, but it's reversible, because by treating with a reducing agent you can remove the additional negative

charge and make it go back to its original position.

Q I'll get to that. But in order for that to occur, do you have to have something of a chemical structure of the particular protein, you have to have a sulfidryl group that can undergo this reaction with glutathione; is that correct?

A That's correct.

Q How many different enzymes can undergo that reaction?

A Well, some number. I don't know what the number would be. Most proteins, red cell enzymes, or many red cell enzymes, have sulfide groups, but only a few that I can think of; ADA and acid phosphatase being the two that immediately come to mind actually undergo that kind of shift.

Q Now, if you used a reducing agent such as Cleland's reagent or mercaptoethanol that will reverse it; correct?

A Correct.

Q Does it reverse it completely?

A Substantially completely. There may be a little bit of a residue of unconverted material, but substantially it is completely reversed.

Q Does anything for any of the proteins that we've been talking about which produces an anodal shift actually occur in the body when the body is alive?

A Yes. Deamidation reactions occur in the body in the normal course of things, and those proteins that are

susceptible of deamidation reactions. Aldolase as I recall is one that is susceptible to a deamidation reaction in the body. The anodal shift does occur in the body as well. PGM is an excellent example of that.

Q So basically what you have in the PGM is two sets of bands, one of which is caused by the actual PGM protein itself, another one is caused by the deamidation of that protein after it's created in the cell?

A Well, possibly caused by deamidation, yes. One is the primary gene product, and the other one is a secondary modification product of the primary gene product.

(No omissions.)

7-1

Q What other reaction can change the presentation of the phenotype on the electrophoretic plate that occur in a wet state besides the bacteria which you mentioned, the formation of mixed desulfides and deamidation?

A You could have neuraminidase from bacteria. You could have proteolytic enzymes from bacteria. Even sometimes they chew up other protein.

Q What happens when you have -- How does that appear when proteolytic enzymes are chewing them?

A Most enzymes, once they are nibbled at, they die. When they are nibbled at, they lose their enzyme activity so you don't see them at all.

Q Do the different phenotypes die due to proteolytic changes at differential rates or do they all go on the same?

A For the markers referred to here, as far as we know, they all die at the same rate.

Q Do those proteolytic changes occur in the dry state?

A No. Again, enzyme reaction doesn't really grow in dry state material.

Q When blood is shed, does the body naturally produce anticoagulants?

A No, it doesn't.

Q Excuse me. Coagulants.

A Yes, it does.

Q And are those dissolved into the blood itself?

7-2

A They are present in the blood to begin with in inactive form. I would -- Well, I will let you follow your line of questioning.

Q When they become active, do they remain active solely in a wet state?

A Yes.

Q Do they have any effects which may alter the presentation of phenotypes on the electrophoretic plate?

A Not that we have been able to ascertain. As the blood dries and it's in the wet state as well, the red cell remains intact, so the enzymes that are present inside the red cell are not really in contact with the coagulation enzymes that are outside of the red cell.

The plasma protein markers are in contact with those enzymes, in the studies that have been done, since the markers that are used survive in bloodstains that have come from coagulated blood, by inference we can say that those markers have not been affected.

There are a number of other markers which don't survive that, so by inference they may be affected by the coagulation in time.

Q Have you been doing studies in that particular area?

A I have, yes.

Q And what about in laboratory samples where anticoagulants are added? Do the anticoagulants essentially stop this coagulation reaction from occurring?

7-3

A Yes.

Q Does that have any effect on the presentation of phenotype?

A Well, it depends on how the sample is stored while in the anticoagulant, and it depends on what anticoagulant is used.

There are some anticoagulants that inhibit enzyme activity. That's the way they exhibit their anticoagulant effect. They can have an effect.

I think sodium flouride is one example, that that's not commonly used for collection of samples for forensic purposes, at least.

Other anticoagulants may have no effect or may even promote the stability of the enzymes. Drawing blood into ACD, acid citrate dextrose, which is essentially the same solution that it is drawn into for storage in the blood bank, preserves the red blood cell and the enzymes in the cell.

Q What about EDTA?

A EDTA can have an effect on enzymes that require metals and that is why red cells that are drawn into EDTA are usually washed before the analyses are done and the electrophoresis is done in a medium that contains some of the metals.

Q Is peptidase an example for such enzyme?

A Yes.

7-4

Q Are there any other wet state changes that we haven't mentioned that affect the presentation?

A Not that I can think of at the moment. Do you have some for me?

Q Let me just check my list.

What are the different changes that an enzyme or protein can undergo in a dry state?

A Most of the wet state changes will not go in a dry state. What can happen in a dry state phenomenologically is that of a loss of solubility of the sample and that is believed to be a consequence of cross-linking of the protein material through a dehydration reaction.

Q When proteins are cross-linked, how does that affect their appearance on the electrophoretic plate?

A It would not because they would not extract. If they are sufficiently cross-linked, they will not extract into solution, so they can't get into the gel. They would not electrophorese.

Q Can proteins also connect with lipids in a dry state?

A Yes, they can. Not at a very rapid rate, but they can through radical reactions.

Some of the cross-linking of the proteins may also be promoted by radical reaction.

Q When the proteins cross-link with lipids, how does that appear on the electrophoretic plate?

A I don't know that we can say anything definitively about

7-5

that because people have not generated chemically defined lipid cross-linked enzymes to see how they would look on an electrophoresis plate.

Q Is there any reason to suspect that they might cause a smearing?

A Yes. That's the most plausible expectation.

Q Why would that be?

A You would have a rather ambiguous change in the charge on the protein -- that is, it would not be a step-like change, but, rather, a blurring of charge.

You would also have a blurring of size. Instead of having molecules that were of a defined size as they are in the native state, by the attachment of the lipid, you would change the way the protein -- you would change the size of the protein and the way the protein interacts with the water in the surrounding environment.

Q How does that affect its electrophoretic mobility?

A Smearing, I think, would be the best explanation.

Q Why is the change in size -- Is it the weight we are talking about, or what parts of the protein are exposed to the outside, as it were?

A Both. It depends upon the medium that one uses. If you have a medium that sieves and the starch is such a medium or acrylamide gels are such a medium, then the protein's molecular weight will affect its mobility in something like that.

In either case, either with sieving media or nonsieving media, how the molecule interacts with this particular environment, with its aqueous environment, may have an effect on its mobility. And this is fairly technical, I should say.

Q The agarose/starch gel that Mr. Wraxall uses in his bloodstain analysis system, with one percent agarose and one percent starch, that is designed, is it not, to minimize the sieving effect in order to promote the resolution of the enzymes?

A The concentration of starch in there is not sufficient to have a sieving effect.

THE COURT: It appears we will go into the afternoon session.

MR. NEGUS: That's a fair statement, your Honor.

THE COURT: Break now?

MR. NEGUS: Sure.

THE COURT: We will return at 1:30. Let's return at that time.

You must return at that time.

(Whereupon the noon recess was taken.)

(No omissions)

SAN BERNARDINO, CALIFORNIA; MONDAY, APRIL 30, 1984; 1:32 P.M.

DEPARTMENT NO. 10 HON. RICHARD C. GARNER, JUDGE

(Appearances as heretofore noted.)

THE COURT: Please continue.

CROSS-EXAMINATION (Resumed)

BY MR. NEGUS:

Q On the electrophoretic plate, we mentioned that it's possible, at least, that streaking could be a result of the lipids --

A Interacting.

Q -- cross linking with proteins. Are there any other causes of streaking on an electrophoretic plate besides the lipids and the proteins getting together?

A None that I can think of really formally. We tend to say things like grunge associated with a protein, and that's why it streaked. That's not really a definitive chemical explanation, but that's about the best that we can do.

Q Is there any particular evidence that this streaking is caused by bacteria appearing electrophoretically on the plate?

A No, there is no experimental evidence which would demonstrate that bacteria are a cause of streaking. Bacteria could contribute some of this grunge, but that

is a guess, not something that has been experimentally established.

Q Is that as good a guess as the lipids cross linking with the proteins?

A I would say that probably there's a lot more available grunge factor, if you will, present in the blood to begin with. You don't really need to introduce bacteria into it. Certainly streaking is seen on bloodstains which are not significantly contaminated with bacteria.

Q The one area where there's at least some evidence that indirectly bacteria may cause streaking is in the neuraminidase and influence on haptoglobin; is that correct?

A I don't know that neuraminidase particularly causes streaking with the haptoglobin. Neuraminidase does have an effect on haptoglobin in that it changes the charge state of the haptoglobin.

Q Doesn't it change the charge state so that different molecules of haptoglobin will sort of spread out and cause what looks like a streak?

A It could, I suppose. I would attribute streaking with haptoglobin to something else, I think.

Q Well, with the haptoglobin technique that is used on that chart, the gradient acrylamide gel, would you expect to get something else that you would attribute it to would be the decomposition of hemoglobin?

A The decomposition of hemoglobin, the interaction of hemoglobin with red cell membrane material.

Q Does that problem occur with the gradient acrylamide gel?

A I don't know the extent to which it would occur with the gradient acrylamide gel. There is a procedure commonly used to minimize the problem, and that procedure is to extract the bloodstain extract with chloroform, which takes a lot of the bound lipid, not covalently bound, but the lipid grunge, if you will, out of the system.

Q That's because the chloroform will make the hemoglobin less soluble and sort of precipitate it out?

A Well, the chloroform makes the hemoglobin less soluble, yes. The chloroform also will extract out a lot of the lipid material. This is something that works empirically and the people who use it attribute it to one thing or another, but it works.

Q Nobody is quite sure exactly why, but for sure --

A There's precedent for both of the explanations I gave you. Chloroform extraction is commonly used in protein purification to selectively precipitate out hemoglobin. Chloroform extraction is also routinely used in lipid analysis to remove lipids from protein solutions.

Q Can an improperly prepared gel cause streaking?

A Improperly prepared in what respect?

Q Can you have any just sort of too heated, for example --

well, when you're making the gel you bring it almost to a boil in preparation of when you're mixing the gel and the starch or the agarose in with the buffer; is that correct?

A Actually you do bring it to a boil.

Q In that particular process can you overheat it and burn it and cause --

A You can overcook it. I don't know whether it would cause streaking or not, because I'm not sure that I have ever overcooked it long enough to bring that effect about.

Q Does a high salt content of your sample, in your sample, cause streaking?

A It can cause streaking. It can also cause distortion of the gel.

Q In what other way besides streaking?

A You mean how does it cause distortion of the gel?

Q Yes. First of all, what does it look like?

A When you have a high salt content, you tend to have the electrophoretic zones warped around the high salt zone.

Q And what causes that?

A You have a higher conductivity where the salt is, so you have instead of a more or less continuous electrophoretic electric field within the gel, you have a discontinuity where the high salt is.

Q Does bacteria cause the type of anodal shift, the same type of anodal shift that you experience with the process that you suspect is deamidation?

A You mean could the presence of bacteria in a sample affect such a shift?
Q Yes.
A I wouldn't say that it has really been documented one way or another.
Q Could the presence of bacteria cause oxidation products?
A Probably not. Oxidation, the formation of oxidation products would be basically independent of bacteria in terms of their chemistry.
Q In a normal PGM run from a dry bloodstain, would you expect to see equal intensity in the a, b, c, and d bands?
A It depends on the type that you're looking at. If you're looking at a 2-1 type, then they would be roughly of comparable intensity, yes, if it is from a bloodstain. If it is from a semen stain, the patterns would be somewhat different.
Q Why is that?
A Because the appearance of the PGM in blood is a little bit different than the appearance of PGM in semen, the appearance of PGM in fresh blood and fresh semen, to start off with. And, say, take a PGM Type 1 as the example, in normal red cells in fresh blood, the a band and c band are of roughly common intensity. In semen the a band is more intense than the c band.
Q That's because unlike the situation in the blood cells, you don't get the same degree of changes from the

phenotype into the second deamidated substance?

A Well, we don't know if it's deamidated.

Q Suspectedly?

A Yes, of the primary form into a secondary form. It's largely a factor of time. The life span of a red cell is on the order of 100 days. So if you sample blood at any particular point in time, the average red cell will be something on the order of maybe 50 or 70 days. I'm not sure what exactly it would be. In any case it's not an adolescent red cell, nor is it a senile red cell. It is probably a middle aged type red cell. And having those two bands of roughly equal intensity is what you see in nice middle aged red cells.

In semen, the enzyme is a much younger enzyme, more like an adolescent enzyme, to keep the metaphor going. And so more of it exists in the primary state than in the secondary state.

Q In the dried red blood cell -- excuse me, dried red bloodstain, will all of those 2-1 bands disappear at the same rate?

A In a dried sample, yes, at approximately the same rate.

(No omissions.)

9-1

Q The what we call on the board there PGM_1 in its primary state is actually two different chemicals; is that correct? There are two different chemicals that it can be?

A What do you mean by PGM_1 in its primary state?

Q The phenotype created that are labeled electric -- that are labeled PGM_1 type, that can actually be two different chemicals?

A Are you referring to the subtypes?

Q Yes.

A Yes. Okay. When in fact it shows up on contentional electrophoresis as a single band, it can be separated by other electrophoretic techniques into two bands.

Q And likewise the two can likewise be separated?

A Correct.

Q The reason for that would be the fact we are dealing with four different alleles?

A Correct.

Q And the subtypes are referred to as plus 1, minus 1, plus 2, minus 2 types?

A Right.

Q Does the 2 plus, the 1 plus and the 1 minus and the 2 minus, are they all equally stable?

A There is nothing, no real hard information on that. There is some feeling that perhaps the minus bands might be a little bit less stable than the plus bands,

9-2

but there is, to my knowledge, only one or two people who have actually put that to the test and they haven't found that to be the case.

So I would say that it's something of an open question. It wouldn't hurt to have some additional scrutiny given to it.

Q If the suspicions of some serologists that the pluses last longer than the minuses is correct, could that result in different rates of disappearance of the 2-1 depending upon whether you had a 2 plus, 1 minus or 2 minus, 1 plus, or other combinations thereof?

A I don't follow exactly your other combinations thereof.

Q Those are the two I was thinking of.

A A sample that has a minus band in it, you are asking whether --

Q Let's take a 2 plus, 1 minus.

A Okay.

Q If the suspicions of the serologist that there is this difference between pluses and minuses is correct, could that result in a situation in the dry blood cell where with the 2 plus, 1 minus, the 2-1 ends up looking like a 2?

A That would be a possibility, I think, but -- or theoretical possibility, but at least insofar as the conventional typing is done, there has been enough testing of PGM involving type 2-1 samples such that

9-3

were this a significant problem, it would have shown up.

Q What about in a situation where you have a wet sample with a 2 plus 1 minus, would that result in a 2-1 looking like a 2?

A I would say that was probably a more likely possibility primarily because the pattern of deterioration or the mechanism of deterioration, I think, is likely to occur more rapidly in a wet state than in a dry state.

But, again, there has not been documentation that I'm aware of that that's a particular problem. One can look at it in the following way.

It's possible that the sun could rise in the west, but no one has ever seen it, so I think that's not likely to be the case. This doesn't have the assurance of the sun rising in the west, when I say I don't think it's a problem, but it has been looked at, this system has been looked at by enough people over a long enough period of time such that if it were really a significant problem, someone should have noted it.

Q Is there any situation with PGMs where suspected deamidation can cause a cathodal shift from the bands?

A Deamidation should not cause a cathodal shift. The deamidation would result in increase in negative charge and that would give you an anodal shift.

Q That's because the particular amino acids which you call amides all have negative charges to them?

9-4

A They do not have the negative charge, but when you remove the amide group, they take on the charge.

Q And in electrophoresis jargon, the portion of the plate which is more toward the origin when you are running PGM's is called the lower side; is that correct?

A Usually, yes, except when one is doing acrylamide gels because in acrylamide gels, the plate generally runs down in the lower portion, the anode.

Q But in the system we are using up there for Group I, that part-starch part-agarose gel and the lower part should be --

A You read it like you read a newspaper.

Q That's the way they always print the pictures in the book?

A That's correct, except when space is limited. Then they print them sidewise.

Q Are there any of the enzymes that we have listed on the board which have recognized typing problems so that rules have had to be formulated about how to go about typing?

A Yes. Acid phosphatase, red cell acid phosphatase.

Q Any others?

A PGM has rules. Actually all of them have rules of one kind of another. For some of them, the rules are more articulated than they are for others.

Q What are the rules for PGM?

A One of the rules of PGM is that you want to have the b band and the c band well separated. If you have good

9-5

separation, good resolution between the b band and the c band -- that is, these two bands -- then you know that you are not likely to make a mistake of confusing a b band and a c band.

And I think that you can see from the illustration that if the b band and the c band merge together, then a type 1 can be confused possibly as a type 2-1.

Q Any other rules for that PGM?

A Some people, I think, apply the rule that unless the a band and the b band are of at least comparable intensity to the c band and d band, they will not make a call.

Q Why is that?

A Because the a band and the b band is of lesser intensity, which indicates that the anodal shift has taken place in the sample that would generally be signaled by additional anodal bands in the position anodal to the d position, called a pseudo d band.

Q How far do you need to stain on the PGM in order to make sure you pick up any bands anodal to the d band that might be significant for typing?

A Well, you mean to apply the rule because it's not really significant for typing?

Q Right.

A Except to the extent that this rule comes into play. If you have good, healthy a bands and b bands, then you

9-6

are okay. If you have a good, healthy b band, for example, with no significant staining in the area of the a band, then you would be disinclined to consider it a 2-1 that possibly has deteriorated to look like a 2.

Q What rules exist for the esterase D?

A The esterase D shows anodal banding as well, and so the rule, the main rule that applies there is looking at the intensity of the bands that one sees.

And if I can illustrate from the figure, a type 1 develops anodal bands roughly in the same position as where the secondary or where the hybrid bands are for the type 2-1. So one has to look at the relative intensities of these bands and compare them to what one expects, the mind's eye picture, the prototypic picture of what a 2-1 looks like.

Q And that's a pattern of intensities with relative order of magnitude are 1:2:1?

A In the 2-1, it's a 1-2-1, although as the type 2-1 deteriorates, you lose a little bit on the 1 and get a shifting toward the anode.

Q In other words, to make sure that you are calling it correctly, unless you see that 1-2-1 pattern, they don't call it?

A Or something that approximates the 1-2-1 pattern. In the 1-2-1 pattern, this band is more intense. As the pattern deteriorates, the anodal, over time, does appear

9-7

to deteriorate such that one of the secondary bands is more intense than the primary bands.

Q Is there also a rule about resolution that some people apply?

A For esterase D?

Q Yes.

A You want to have your bands well delineated. With the multisystem, there is separation between the secondary bands and the hybrid bands, so one would like to see them separated as well.

Q And if they become diffuse, then it's considered, then, not readable?

A If it's too diffuse, many analysts would not consider it readable.

(No omissions.)

Q Do any of the bands in the esterase D inactivate at faster rates than the others?

A Well, given the general shift that esterase D inactivates through an anodal shift pattern, the slower band, the band that is seen in the Type 1 pattern, or this band in Type 2-1 pattern, would appear to be decayed or rapidly decaying.

Q Is it possible that in the 2-1 pattern that the secondary band would inactivate at a faster rate than either the 1 or the 3?

A What do you mean by secondary band?

Q The band in the middle.

A The hybrid band?

Q The band in the middle.

A The hybrid band.

Q Right.

A No. The hybrid band appears not to deteriorate faster than the 1 or the 3 band.

Q In the acid phosphatase, what rules have been developed by yourself and Mr. Wraxall to type acid phosphatase?

A Well, first you want to have adequate resolution. Acid phosphatase isn't really illustrated there.

Q Would you like to go and --

A I would be willing to do so.

Q Why don't you do it -- yeah. The first chart that you did this morning on direct, could you label that PGM?

A (The witness complies.)
Q Then if you would -- you've labeled PGM 1 to be from one gene locus and the other one to be from the other gene locus?
A Right, bacterial. That's what that's supposed to represent.
Q At the top?
A Yes.
Q So if you could draw the patterns that you get in an acid phosphatase.
A There's the six common phenotypes.
Q You were going to use that illustration why you need good resolution.
A Okay. Want to have resolution between the fast b band and the slow a band, up being the direction of migration.
Q Up being the more up it goes the faster it is?
A Towards the anode, yes.

Okay. So you run appropriate standards so that you can make certain that you have good resolution there. That's one consideration you would like to see. In typing any sample that involves an a, you would like to see the appearance of this minor band. And the reason for that is that for both a types and b types, the faster band appears to deteriorate at a more rapid rate than the slower band, the reasons why being point of biochemical controversy, which are not really relevant in

terms of the phenomenology. In any case, this band may disappear before this band does.

Q The faster band disappear before the --

A The slower band. So if you hold off on making judgment, say in a B A type sample, until you can see whether anything shows up here in the slower a band position, then you are not likely to make a mistake.

Q So the two rules essentially involve the use of the proper standards in order to make sure you have resolution and making sure you have slower bands as well as the faster bands before you make any typing call?

A You would need another standard. You ought to have a C B standard. The only way that Type B or Type C can be distinguished is that the two types differ in staining intensity of the bands, and because of the phenomenon described over time, that the faster band tends to go up more rapidly than the slower band, one can get a confusion of B and C. This is well documented in the literature. This is essentially a wet state reaction and it occurs at the rate of 24 to 48 hours at body temperature in the blood.

Q What then standards do you need to use in order to make sure that you have a proper resolution on all the different bands? Do you need three different ones?

A I like three. I like to use the A, B and C B. There's another reason for having the C B on there, and this

is if the plate heats up in the course of the run, then your B pattern may look like a C B, your C B may look like C. So by running those two standards on the plate, you can tell whether you have had a heating problem.

Q Would just using a B A and a C B satisfy your criteria as a standard?

A They would, but I prefer the other as a point of preference.

Q Why is that? I mean, why do you -- what's the difference between a B A and a common -- and then using a B and an A separately?

A Well, the B A is a better standard in that you have a comparison of these two, the mobility of these two bands in the same track.

Q So you prefer to use B A rather than the A and B?

A No. I'm saying the B A would be superior with regard to judging resolution.

Q Oh, okay.

A But the B and C B would be superior with regard to the intensity problem.

Q So if you used the combination of B, B A, and C B, that would be the best of all possible combinations?

A That would be a good combination, yes.

Q You mentioned problems that can be caused with acid phosphatase of heating up during the course of a run. Does that mean that just in the course of actually

analyzing the acid phosphatase you can create through excessive heat some of the same wet state changes?

A Yes, or at least that is presumed to be the case. I'm not sure that it has been documented in a hard scientific way, but that is believed by most people in the field, I think, to be a possibility.

Q That would explain getting wet stain type changes when you actually have a dry stain that you're analyzing?

A Yes.

Q Are there any rules that have developed for the ADA?

A The principal one is, to be sure, and use a reducing compound when you're doing the analysis.

Q That's to get rid of the mixed disulfides?

A Right.

Q Any others?

A To the extent of my knowledge, I am not aware of them.

Q Any other rules, any rules for AK?

A Yes. You look at the extent of anodal band formation with adenylate kinase.

Q Why is that?

A Because the adenylate kinase, when it deteriorates, forms an anodal band. In looking at this sample, the Type II sample, the anodal band of the Type II, the deterioration band of the Type II, is in the same position as the Type I band.

You see this generally as a stack of bands, so you

look at the extent to which you have that anodal band. If you don't have very much anodal band, then you are not concerned about the typing as the amount of anodal banding builds up. If you have a suspected Type 2 or Type 2-1 sample, then you become more and more cautious.

Obviously, for a Type 1 sample anodal bands are not going to affect the general typing interpretation.

Q What rules do you use in interpreting transferrin?

A With transferrin, you look at the multiplicity of bands that you see. The principal thing that you would be concerned with with transferrin would be desialidation; that is, the removal of sialic acid by neuraminidase. And there you would see if neuraminidase has been active on the protein. You would see cathodal bands. And anywhere from two to five bands would show up with your transferrin stain.

Q Is it possible to eliminate those bands by treating the whole sample with neuraminidase and shifting the whole pattern to the cathode?

A It is, or in the cathodal direction, yes.

Q What typing rules do you use for Gc?

A Good resolution as always. Good resolution is the necessary ingredient for all of the electrophoretic typing. Beyond that, Gc typing is pretty straight forward, pretty non-ambiguous.

There is the formation of an unusual kind of anodal

band with Gc, something that's not a one step away band, but rather a block away, if you will, on the spatial relation of the typing gel. That mirrors the standard pattern and indeed one of my colleagues has suggested that you just have to push Gc so that it all shows up in the anodal position, and you can type it unambiguously in the anodal position. That has not met the test of reliability yet, but --

Q Generally, if you get this pattern and a block away from Gc, it's not called?

A No. I wouldn't say that it's not called. It depends upon how much you have in the standard band position, what you see in the standard position.

Q Do you sometimes get a situation where the whole thing is moved up a block as it were?

A Yes, yes, you do. And as my colleague says, when you see that you can type it that way, but that has not yet come into standard practice by the people in the field.

Q In applying all of the rules in order to make sure that you have reliable results, is it critical that the analyst be prepared to say that a particular electrophoretic run results in a -- is it important in order to ensure reliability that the analyst be prepared to say I can't type it if in fact there's any ambiguities?

A Yes, indeed. The most critical judgment to make is the judgement that something is not typable.

Q And is it acceptable to say, well, it sort of looks like that, and therefore it probably is~~n't~~, so I'm going to call it, or should you just say I can't type it?

A Well, what people actually do, I can't speak for them. But most people, if they err, they err to the side of being conservative. They will not call plates or samples which possibly could be called.

Q If they are calling ones that shouldn't be, then that can affect their reliability?

A You're speaking of their reliability as an analyst now?

Q Yes.

A If they call ones that they should not be calling?

Q Right.

A Yes, they are running the risk of error.

Q What rules have developed for the typing of peptidase A?

A Peptidase A is not a marker that I can describe rules for in great detail, because my laboratory has not done very much with peptidase A at all, and I have not been involved with the development of those rules.

Q What about CA II?

A CA II is pretty unambiguous. If you have good resolution of your bands, then in my experience there is not a problem with this typing.

(No omissions.)

11-1

Q Does CA II appear in semen?
A Not that I'm aware of.
Q If you were to, on semen, using the Group IV approach on the diagram there and you got a result of CA II 1, something you called the CA II 1, what would that be?
A I'm not entirely sure. It could, as I say -- As I say, I don't know whether semen has carbonic anhydrase in it. I have not done testing for it, so if carbonic anhydrase is truly there, then I would say that it's probably CA carbonic anhydrase type 1.

If, on the other hand, carbonic anhydrase should not be there, then I would have a great deal of wonderment as to what the carbonic anhydrase staining might be.

Q Are there any rules that have been developed for the calling of haptoglobin in using the gradient acrylamide gel?
A Some general rules. Because of the way haptoglobin is stained, you would like the residual hemoglobin in your sample to stain away from the haptoglobin bands region so you do not have a large smear of hemoglobin that smears your haptoglobin pattern.
Q By making sure of your range of gradients like 4 percent to 30 percent?
A That's one of the approaches that's used. To look at the electrophoresis that way, the hemoglobin, most of the hemoglobin ends up migrating to a position that's

11-2

separated from the haptoglobin band position.

Q Any other rules for haptoglobin?

A For haptoglobin typing, you ought to have good standards, preferably have all three types of gel, although you can get by with a 2, 1 and 2, as your standards for typing, calling a sample a 2-1. You ought to see a reasonable 1 band and a multiplicity of bands that you see toward the top of the gel should line up with the bands in your 2-1 standard.

Q Does using the gradient acrylamide gel, does the electrical charge of the hemoglobin complex make any difference as to where the protein ends up?

A If you use a gradient that's sufficiently steep.

Q Like from 4 to 30?

A 4 to 30, yes. And you run it long enough, you essentially get to a steady state position or a steady state pattern where the protein runs into a thickness of gel through which it cannot penetrate and its mobility halts so that essentially becomes a separation based upon molecular weight rather than based upon charge.

Q So even if you have a diffusion in charge among different molecules, same weight, they are going to end up at the same spot?

A Approximately the same spot, yes.

Q In terms of the biochemistry of the proteins, other than haptoglobin, what has changed in each of them is amino

11-3

acid which has resulted, a different amino acid which has resulted in a different electrical charge; is that correct?

A Yes.

Q Haptoglobin is different?

A Yes, haptoglobin is unique.

Q How is that?

A The mutation such as it is did not result in the replacement of one amino acid by another amino acid in the polypeptide chain, but, rather, of a polypeptide chain which nearly doubled in size. One piece of polypeptide chain tied to the other end of another piece of polypeptide chain, one piece of gene tied onto another piece of gene, the net result being the polypeptide chain in the type 2 type which is nearly twice as long as the polypeptide chain in the type 1 type in which the amino acid sequence and the front half and back half is near duplicate.

For the one you would have the alphabet A to Z; then the other, you would have the alphabet from A to R. Then it begins at C and runs through to Z.

Q The 2-1 type has a combination of both your A to Z then your longer alphabets?

A That's right.

Q The type 2-1 modified, it's the 2 that's modified; is that correct?

11-4

A Yes. It appears to be produced at a lower level than the 2 normally is.

Q How does that reflect itself using this gradient acrylamide gel?

A You tend to see a greater preponderance of the lower molecular weight forms as compared to the higher molecular weight forms.

Q So you will get a --

MR. NEGUS: Could I have another piece of paper?

THE COURT: Yes.

MR. NEGUS: Which will be marked, I take it, as K-9?

THE CLERK: Yes, it is, your Honor.

Q (BY MR. NEGUS) Could you draw a 2-1 and a 2-1 modified pattern?

A These are going to be very approximate.

This would be, I hope, a reasonable approximation.

Q The heavier are toward the top?

A Yes.

Q Now, when you have a weak sample of a 2-1, do any of those particular bands not show up first?

A Yes. This part.

Q Toward the top?

A Toward the top tends to be the weaker part of the pattern.

Q So if you have a weak 2-1, it can end up looking like a 2-1 M?

A The band intensities would not be quite the same. With

11-5

the 2-1 M, the band would be much -- With the 2-1 M, the 1 band would be much more prominent in the 2-1 M sample than in the 2-1 sample.

Q The difference between a weak 2-1 M and a weak 2-1, would they end up looking pretty much the same?

A No. Probably not. Again, because of the relative intensities of the bands through the middle range and the major bands at the bottom.

With the major band at the bottom is the known polymerized molecule, the haptoglobin which consists of two alpha 1 chains and two beta chains, and the proportions of the bands that one sees throughout in a 2-1 sample depends upon the starting proportions of the alpha 1 chain and the alpha 2 chain, and if they start out at nearly equal proportions, then one tends to see a band, an alpha 2, beta 2 band, which is anywhere from weak to moderately strong, and then a series of polymeric bands above it of higher molecular weight band.

With the 2 M, since the alpha 1 chains are present in much higher proportion to begin with, it's clearly the dominant band and one sees the polymer bands at the lower intensity.

Q In order to be confident in calling a 2-1 M, you need to have a certain intensity to the 1?

A Yes.

11-6

Q And is that something that's quantitatable or something that's based upon experience?
A It's quantitatable, but it's based primarily on the experience.
Q How would you quantify it?
A I would use an entirely different technique than this. I would separate the polypeptide chains and separate them on the basis of molecular weight and then look at the relative intensities of the alpha 1 chain and the alpha 2 chain.
Q But just in order to make a reliable call using the 4 to 30 percent gradient of acrylamide gel, it's essentially just a question of knowing how intense it has to be before you call it?
A That's right.
Q In the PGM_1 where you have subtypes, is that referred to as a hidden variation?
A When you subtype.
Q The subtyping is done by isoelectric focusing?
A Either that or electrophoresis under different pH conditions.
Q Before that was done, it was just generally thought that all 1's were 1's; is that right?
A That's correct. 1's still are 1's. It's just that you can subdivide 1's into 1 pluses, 1, and 1 minuses.
Q Basically before that was discovered, the 1 plus,

11-7

1 minus, was anybody aware that there were two different chemicals that were going by the same name of 1?

A No.

Q That phenomenon where you have two different chemicals going by the same name, because they appear at the same spot on the electrophoretic plate, is that known as a hidden variation?

A Hidden variation or cryptic variation.

Q Are there any other of the proteins that we have been considering that have hidden variations to them?

A Esterase D does and haptoglobin does.

Q What about peptidase A?

A Yes, peptidase A does. It's not accessible the same way the others are. In red cells, peptidase A is present as an activity variant. Some people have high levels of peptidase A activity in their red cells; others have intermediate levels; and some people have low lvels.

After a fair amount of searching for the electrophoretic system, one was found that would enable the high activity form of the enzyme to be distinguished from the low activity form of the enzyme.

Q That's 1 from an 8?

A That's correct. The problem is not everyone has been able to get that to work reliably, and so apart from a very few publications in which the 1 and 8's have been differentiated electrophoretically, it has not been

11-8

given nearly the scientific play, if you will, as that of the differentiation between the 1 and 2 has been given.

(No omissions.)

Q Do the 8 and the 1 show up in the same spot?
A Yes, essentially the same spot.
Q And certainly they show up in the same spot using Mr. Wraxall's multisystem?
A Yes.
Q The 8's and the 1's have differential survival rates? Does one last longer than the other?
A In lipid or in red cells, it appears that there is no difference. It was suggested initially, that is when the activity variation was first found, that a plausible explanation for the activity variation was that there was one form of enzyme that was stable to Type I and another form of the enzyme which subsequently came to be called Type A or the 8 isozyme. The studies were done in red cells looking at the adolescent, middle aged, and senile red cells, and it was found that the change in activity that occurred over the life span of the red cell could not account for the differences that were seen. So that hypothesis, that is, that the 8 represents an unstable form of an unstable enzyme, I think has been basically rejected.
Q Nobody knows whether the 8's last longer than the 1's or the 1's last longer than the 8's?
A Right. Remember the 8's started at a much lower level, so even if they were to disappear at the same time, the 8's would disappear first because there was a lower amount

of them to begin with.

Q Would the hidden variation in the esterase D as the 5 and the 1 --

A The 5 and the 2.

Q Do they -- does the 5 have a different stability than the 2?

A It appears not to. There is one paper that I'm aware of that suggests that it might.

Q What's that?

A I think that would be a paper by Sutton, which appeared in the Journal of Forensic Science Society in the last year. I'm not sure from that paper whether he has suggested the 5 is less stable or that the 5 and the 2 have altered stability or whether the reliability of typing using isoelectric focusing, which is the way you have to -- the technique you have to use in order to distinguish the 5 and the 2, whether the reliability of focusing as a technique would be good for stains more than a couple weeks old. I don't have a clear recollection of that paper, so I can't comment in a hard way to the content of it.

In any case, Sutton's typings have been refuted. Other people find that isoelectric focusing can be used to type the esterase D subtypes in stains older than the stains that Sutton was able to type.

Q Has that work been published?

A It is due to be published. It has been presented at one, and I think two meetings, and is due to be presented again next year at the international meeting.

Q What are the hidden variations in haptoglobin?

A The hidden variation in haptoglobin is in the Alpha 1 chain, and in order to detect that variation you have to chemically disrupt the protein, do a chemical modification of the protein, so that it does not spontaneously reform, recombine, and then do an electrophoretic analysis.

Q That involves an entirely different technique than was used in this particular chart; is that correct?

A Yes, yes.

Q The one subdivides into one fast, one slow?

A Correct.

Q Does the one fast and the one slow have a different stability from one another? Does one last longer than the other?

A Not that anyone is aware. Again, this is the kind of problem that if it existed, enough haptoglobins have been looked at it would have been seen. What's more, it's probably irrelevant in terms of the typing of haptoglobin.

If you have a one fast, one slow type, then since by conventional typing methods they are indistinguishable, you would see it as a simple loss of activity situation.

In a Type 2-1, since the patterns of the 2-1's are so strikingly different than the patterns of the 1's, one would see some indication in the polymeric pattern formation that is in the higher molecular weight bands, even if the Alpha 1 or the band that contains the unpolymerized material were of weakened intensity.

Q You mentioned that heat can cause wet state changes to plates and dried acid phosphatase. Can heat cause any other changes in the presentation of the phenotypes of any of the proteins that we've been talking about?

A Okay. First, it wasn't quite the way you stated.

Q Okay.

A Heat can cause changes in acid phosphatase when it is in solution after it has been extracted from a bloodstain. In the dry state proper heat seems not to have a significant effect on the band patterns.

Q I didn't mean to suggest otherwise.

A Okay.

Q Does heat have effect on any of the other proteins?

A The principal effect of heat is to inactivate them.

Q What about an improper cooling of the gel, which I take it is what usually causes the problems with acid phosphate.

A If the gel gets too hot, you'll simply inactivate the protein.

Q What if it's just a little bit too hot?

A You will inactivate some of the protein.

Q How can you tell if that's happened?
A It would generally show up as a weaker staining intensity on the gel.
Q Do you ever get -- does heat ever cause like warping?
A Yes, heat can cause warping.
Q Does that affect the reliability of that particular gel?
A It depends upon the degree of the warp. If the warp is substantial, then, yes, you'd probably want to throw the gel away and not admit to anyone that you ever ran it. On the other hand, if the heating problems are mild and the pattern of the warp can be followed, then you can make a reliable typing from that.
Q How do you tell the difference between a substantial warp that you'd throw out and a mild warp that you don't? Just experience?
A Basically experience, yes.
Q In making sure you don't have heating problems, there's a cooling system that's used in most electrophoretic plates; is that right?
A One doesn't have to use a cooling system. If you want to run at high voltages, then you need to have a cooling system. If you want to run at lower voltages, then you can do the electrophoresis in the refrigerator. That provides ample cooling. If you run it at lower voltages, you can run it at room temperature and you will not get significant heat buildup.

Q When you do use the cooling system, there's sometimes a cooling plate which is cooled by water or some other form, and then the glass plate is put on top of that?
A Yes.
Q Just as a matter of thermodynamics, which is more efficient: to have air between the glass and the cooling plate or water?
A Water between the glass and the cooling plate. That would give you better thermo conductivity. And you usually do have water in there, because there's condensation on the surface of the cooling plate.
Q It wouldn't make sense for the efficiency of the system to keep wiping off the glass plate to get rid of the condensation, right?
A Well, you wipe it off so that you don't have excessive amounts of moisture on there. Otherwise you'll have basically a short circuit of your gel. So you need to have -- you will have some condensation there, but you don't want excessive amounts of condensation.

THE COURT: Is this a good breaking point, Mr. Negus?

MR. NEGUS: Sure.

THE COURT: Let's take a 15-minute recess.

(Recess.)

(No omissions.)

13-1

MR. NEGUS: Your Honor, just one other thing. We would request this one time only. Never again.

Dr. Sensabaugh has to get back to Berkeley tomorrow afternoon. We would like to stop at 4:00 so I can get my transcript, but I would like to start at 9 tomorrow. We probably will be finished by no later than 12:30 for the day.

THE COURT: As a matter of fact, I just spoke to my clerk. I could start earlier tomorrow.

MR. NEGUS: 9 will be just fine.

THE COURT: No problem.

Q (BY MR. NEGUS) Does the -- I think the technical word is substrate on which a bloodstain is deposited affect your ability to type it reliably?

A For the most part, no. You are referring to the enzymes?

Q Right.

A Yes. For the most part, no. Bloodstains or markers in bloodstains survive a little bit longer, it appears, on some materials than on other materials. But there appears the survival is a different matter than typing reliably.

Q Do some substrates wipe out your ability to type altogether?

A Conceivably if you were to drop a drop of blood into an acid solution, that would wipe out the markers. But on most things like tile or fibers, glass, painted surfaces, things of that sort, the surface does not

13-2

seem to have much effect, at least with regard to our experience.

Q Now, with respect to enzymes, does blue denim of the kind that's commonly found in blue jeans diminish the ability or wipe out the ability -- Strike.

Does blue denim wipe out the ability to type enzymes?

A Not in my experience. I have prepared stains on blue denim and have been able to type them or have them typed by students, so it does not seem to be a problem, at least to the limited extent of my knowledge.

Q What about nylon?

A No. Theoretically on dried stains over very long periods of time, you could get a cross-linking between the protein material and the stain in the nylon, but I think that would have to be over quite an extended period of time, and that would not affect typing reliability. That would affect survival.

Q That would be a period of months rather than days?

A Months to years rather than days.

Q Is there in the literature reference to blue denim affecting ability to do ABO typing?

A I'm not sure that it's in the literature; however, it's anodally known that blue denim can cause problems.

Q Why is that?

A Presumably -- and it has not been pinned down, to my knowledge -- but presumably it's because of the sizing

13-3

in the denim material which has some ABO-like substance in it, something that gives a background reaction with the enzyme.

Q Something in the shape of the denim, molecules similar to the shape of the enzyme?

A No, not in the shape of the denim molecules. I would guess it would be in the sizing material. The material, when it's freshly prepared at the mills, I guess, has often a starchy-like material added to it to give it a little bit more substance. That gives way after numerous washings, sometimes after not very many washings, which is why that nice, stiff shirt that you bought suddenly turns into a limp rag.

In any case, the sizing material presumably contains carbohydrate structures that are similar enough to the ABO antigenic structures so that the antibodies can recognize them.

Q So what happens is this starch stuff will bind the antibodies in the same way the antigens do?

A Yes.

Q Is there anything known about this starch that could interfere with protein typing?

A No.

Q Aside from the peptidase A and the PGM, can any other of the polymorphic proteins that we have been considering be determined through semen?

13-4

A If you have semen at sufficient concentration, you may be able to get transferrin typing.

Q What about GLO?

A Yes, it's present in the semen. If the semen sits around for a period of time, the glyoxalase has a tendency to deteriorate.

Q How long is that period of time?

A It's not well established. Liquid semen stored at 37 degrees on the order of -- that's 37 degrees Celsius, body temperature -- on the order of four to eight hours, I think, shows a significant deterioration of glyoxalase.

Q What if it's dry?

A If it's dry, it's stable.

Q How long does it normally take semen to dry if you just deposit it on a blanket?

A That depends on a couple of variables. One variable is rate of air motion around the stain. Another variable is the surface area of the stain. A third variable is the relative humidity of the atmosphere.

In Boston in July, things don't get dry. That's where the relative humidity is close to a hundred percent.

Q Assuming that the humidity is pretty much like it is in this particular room, what is the range that you can have with the other variable?

A Standard indoor humidity runs between 45 and 60 percent. At least, that's what it has been running in my office

13-5

for the last year or so.

With a reasonable amount of air movement, a small stain could be dry in half an hour to 45 minutes. A large stain might take up to two hours or so to dry.

Q If the glyoxalase did become unstable, would it become unreliable?

A It has a tendency, glyoxalase has a tendency to go, off of the limited experiments that have been done and, unfortunately, never follow up on, to indicate that there is something of a band shift as it deteriorates.

The band shift, as I remember it, would be of a sort that would signal itself that something strange is happening.

(No omissions.)

Q So you wouldn't be likely to miscall; just wouldn't call?
A Correct.
Q The experiments that you've done in your office where the relative humidity is ranged from 45 to 60 percent, that's at the University of California in Berkeley?
A Correct. The experiments aren't done in my office. My office is at 45 to 60 percent relative humidity.
Q Okay. Well, you've tested your office for --
A I have a humidity meter next to my desk.
Q What's the external relative humidity in Berkeley?
A Well, when it rains, it's a 100 percent. When it dries and we have Santa Anas, it gets down to 10 or 15 percent. When it's raining, the relative humidity in the office goes up to about 60 percent. So even those there's a high relative humidity outside, inside the relative humidity doesn't change as dramatically.
Q When it's 15 percent outside, is it like 45 to 60 percent inside?
A When it's 15 percent outside, it tends to be on the order of 45 inside.
Q Is the inside relative humidity partly a function of the space? In this room you get less buildup of relative humidity than you would in a smaller one, for example?
A It depends upon the air movement in the space as much as anything. I wouldn't want to predict with the air

conditioning system in this building what relative humidities might be. The air conditioning system probably would regulate the relative humidity pretty -- within fairly narrow limits.

Q Assuming that offices at Berkeley haven't changed much in recent years, I take it it's a small office?

A Actually it's a large office as offices at Berkeley go.

Q Approximately what are the dimensions?

A It's about 200 square feet.

Q In a tight hall or closet, the humidity is going to go up?

A Depends upon, again, upon air flow, it depends upon where the air is coming from. If air is blowing in off of a puddle, say, then the humidity might be higher. If it's blowing in off the desert, then I would guess the humidity would be lower. In a closet, there isn't really a source of moisture. There is in an office a source of moisture. Wherever there are people, there's a source of moisture.

Q A person in a closet would produce a source of moisture?

A A person in a closet would produce a source of moisture, yes.

Q Can a pool of blood raise the relative humidity?

A It would depend, then, as you correctly asked earlier, upon the air space available around it. A one-foot square of blood over here on the floor would not have

much of an effect on the humidity where you were standing. There might be localized changes there. The blood would probably get dry before -- without having any appreciable effect on the humidity of the room.

Q In a house could one expect greater humidity in a hall outside a bathroom than, say, an open living room?

A If someone is taking a shower in the bathroom, possibly, yes.

Q How long would those effects last; do you know?

A No, I don't. You could probably get a rough estimate on it. When you take a shower in your bathroom, your mirrors fog over. How long does it take for the mirrors to clear up?

Q Do any of those polymorphic proteins exist in saliva?

A If they do, at a level that would not be practical for typing purposes.

Q Is there any other genetic marker that's polymorphic that exists in saliva that you can type?

A There are genetic markers present in saliva, a half dozen of them or so. They have either been -- let's put it this way: the paternity people who deal with fresh material are not comfortable typing them for paternity purposes, or at least the paternity people I know. And this is because basically the typing of salivary markers is done in only a few laboratories, and not everyone feels comfortable with the typing that

is done. The typing in stains has not to my knowledge been documented as a reliable procedure.

Q What about with amylase?

A Amylase? Amylase does show genetic markers. Salivary amylase, the frequency of the variant types is pretty low, and one that has a low payoff of yielding useful genetic information.

Q Is it higher amongst blacks than it is amongst Caucasians?

A I couldn't give you an answer on that. I don't know what the gene frequencies are for the amylase.

Q Can you reliably type Lewis antigens from saliva stains?

A That's a point of contention, I believe. Some people do Lewis typing from saliva stains. Other people don't feel comfortable doing those types of saliva stains.

Q What method do those people who feel comfortable use? Absorption-inhibition or --

A No, they use an agglutination-inhibition typing technique.

Q Is there a difference between absorption-inhibition and agglutination-inhibition?

A They are used by some people synonymously, but I don't use them synonymously.

Q To your mind what is the difference?

A Absorption-inhibition is the technique that one uses for typing bloodstains, and it is analogous to absorption-elution in the sense that antibody is absorbed and then you measure -- in the case of absorption-elution

you measure the absorption of antibody by its removal from the general antibody solution. Agglutination inhibition measures the presence of soluable substances in a liquid material or in a stain extract.

Q You indicated that amylase is present in high concentrations in saliva?

A Yes.

Q Are there any units for expressing the volume?

A There are several units that have been defined. I can't give you standardized units. There are several standardized units that are used in the clinical literature.

Q What is the approximate difference in, say, the amount of amylase present in saliva and the amount of antigens, ABO antigens, present in saliva?

A What do you mean by difference?

Q Are they present in the same amounts or --

A Oh, well, they're apples and oranges. Antigens are measured in terms of titer units usually. Amylase activity would be measured in terms of enzyme units. Usually when one does an amylase test when testing for saliva, one does a semi-quantitative test in which you look to see how fast the reaction goes within a certain time interval. If the reaction has gone within that time interval, you consider it a positive reaction.

Q When you are measuring -- excuse me. When you are trying

to determine the presence of amylase in suspected saliva stains, generally the substance that you think is saliva is dissolved in a saline solution first?

A You can do what is in effect an in situ spot test. You can swab a little bit of the suspect material with a piece of filter paper that is impregnated with an amylase indicator solution, so that if the amylase is present then the filter paper turns a color or loses a color. That's one kind of test.

Another kind of test would be as you described it, you would prepare an extract of your suspect stain and test the extract for amylase activity.

Q When you're preparing that extract, is there any way to determine what the dilution of saliva to saline is?

A Oh, okay. You can get a rough estimate, yes.

Q How do you do that?

A If you do a quantitative assay, if you know the range of saliva or -- excuse me, the range of amylase that is present in liquid saliva, then you can take the measured amount of amylase in your stain extract and extropolate, if you will, a minimum dilution and a maximum dilution based on the knowledge of the amount that is normally present in saliva to begin with.

Q So in order to do this quantitative assay, you would have to essentially compare how much starch the undiluted saliva eats up in a given point in time with how much

starch the diluted saliva eats up in a given point in time?

A That's one of the assays for amylase. There are many others, but that's one, yes.

Q If you do the quantitative assay, what is the maximum dilution of saliva to saline that you can have and still be confident that a negative result on an agglutination-inhibition test indicates that you've got a non-secretor?

A That would depend upon the method that you used for the testing of the secretor substances. If you use a relatively insensitive method for typing the secreted substances, then you could not have a very large dilution, on the order of one to ten, say, before it would be impossible to distinguish between a non-secretor and a -- non-secretor saliva and a saliva that contained secretion at low level.

If you used a more sensitive procedure, then you could go to a greater dilution. It depends upon the sensitivity. In short, it depends upon the sensitivity of the procedure that you used for the ABO blood group substance.

Q What is sometimes referred to as an absorption-inhibition technique for determining the ABO antigens would be a relatively insensitive technique; is that correct?

A No, no. It can be quite sensitive. It depends upon the parameters of the test that is used. In some people's

hands using a microscopic inhibition of agglutination -- that is, using a microscope to detect the inhibition of agglutination -- it can be quite sensitive. If you use just your eye to detect the inhibition of agglutination, then it is somewhat less sensitive by a factor of about 5.

Q So if you were to use a microscope, you can get it down to where you could be fairly confident with, say, a dilution of 1 in 50?

A Yes. I would say in that range. This all has to be a little bit hypothetical, because it is predicated also upon knowing what titer range of ABO substances would normally be found in the saliva given that particular technique of substance detection.

(No omissions.)

15-1

Q Are those figures known?
A They are in a not very complete way. And by that I mean that some people have been very much concerned with this issue. In using their techniques, they have developed this information. Other people have not been so concerned with the quantitative aspect of it and if they use different techniques, it would be different to extrapolate exactly what ought to be expected using their techniques.
Q So if you are going to use the agglutination inhibition technique to reliably predict nonsecretor status, you are going to have to know what your titers are?
A That's right.
Q Amylase itself will show up at dilutions considerably greater than blood in antigens; is that correct?
A Yes.
Q So unless you do these quantitative assays and have known titers, merely getting a positive test for amylase and negative result on your agglutination of inhibition test doesn't tell you whether you have a nonsecretor?
A Correct. You have to have additional information.
Q Does nasal mucus contain polymorphic proteins?
A I would presume it does, but I wouldn't know what they would be. Electrophoretically proteins?
Q Correct.
A Yes.

15-2

Q Do epithelial cells contain polymorphic enzymes?
A Yes, epithelial cells are basically cells and they contain the enzymes that other cells contain, so they would have most of the enzymes that are necessary for the metabolic operation of the cell.
Q That would include peptidase?
A Perhaps not a peptidase. It would include some peptidase, but perhaps not peptidase A. It would certainly include PGM since it seems to be one of the essential enzymes.
Q What about esterase D?
A I would guess probably so.
Q And CA II?
A I don't know about that. I wouldn't hazard a guess.
There are other carbonic anhydrases and those cells, epithelial cells, may show others.
Q Would those other carbonic anhydrases show up at the same spot on a Group IV electrophoretic plate as a CA II 1 band would?
A No. Should show up in a different position, and they also have a different substrate specificity -- that is, different staining procedure issues for their detection.
Q Would the same, if esterase D is present in the epithelial cells, would the same electrophoretic setup as is on K-1 be usable to type it?
A Yes.

15-3

Q Same for the PGM?
A Yes.
Q And same for the PEP A, if in fact there is PEP A?
A Yes. You would have to have a sufficient volume of PEP A cells in order to do the testing, and that's a requirement. If you didn't, it's just like the bacteria. If you don't have so much of them, then you won't be able to detect their presence.
Q But if you have, as it were, a big nose blow into a napkin and you get the results, they should be reliable results?
A I don't know how much, what the volume of epithelial cell is in a big nose blow. My experience is mostly it's mucus.
Q How would you test to see whether there were epithelial cells of any quantity in a stain?
A In a mucus stain?
Q Yes.
A With a smear of stain, I would look at it under the microscope and probably do some cell strain which would show up the structure of the epithelial cells.
Q If you saw a lot of them, then you would probably type it?
A If I saw a lot of them, I might attempt typing, yes.
Q These electrophoretic techniques are used to determine the presence and types of polymorphic proteins in not just semen, saliva and blood stains; is that correct?

15-4

They are used for stuff taken from lungs, livers, spleens?

A Yes. Just about any tissue material you can electrophoretically type something.

Q The only reason that in the forensic context there is heavy emphasis on semen, saliva and blood is that is what you generally get in evidence?

A Semen and blood, and mostly on blood. As I stated earlier, saliva poses problems.

In postmortem material, you are sometimes faced with other tissue problems.

Q And in determining the identity of people who are deceased, that kind of typing has been carried out in forensic context for some period of time; is that correct?

A Yes.

MR. NEGUS: I think this is probably as good a time as any.

THE COURT: All right. We will adjourn until 9:00 tomorrow morning.

Please return at that time.

(Whereupon the proceedings taken and had on this date were adjourned.)

--o0o--