Addendum I 270

The Commonwealth of Massachusetts

Department of Public Safety

1010 Commonwealth Avenue, Boston 02215

May 9, 1972

LAB.NO. 38039 - Examination of Materials in connection with the Fatal Beating at Chicopee on April 15, 1972. Victim: Daniel Croteau, 106 Ferncliff, Springfield.

On April 18, 1972, Trooper James Mitchell of the State Police Detective Bureau delivered the following items to this laboratory in connection with the above subject:

1. Decedent's clothing
2. Soil from probable location of struggle
3. Soil from tire imprints
4. Soil from location of body
5. Soil from near tire imprints
6. Stones
7. Stained paper
8. Decomposed blood from south side of river
9. Plastic straw and cotton rope from river bank
10. "Certs" gum and gum wrappers
11. Piece of newspaper

These items, submitted by Detective Lieutenant James Fitzgibbon of the State Police Detective Bureau and Captain Edward Rojowski of the Chicopee Police Department, were to be examined for human blood, blood group and evidential traces.

<u>EXAMINATION</u>

<u>Item 1 - Decedent's clothing:</u>

A. <u>Beige suede jacket "TeeGee, RN 36021"</u>; A 8" x 6 1/2" piece is missing from the lower front. A portion of the lining is partially torn away. A tear was also noted on the left sleeve near the cuff.

Traces of blood were indicated by positive benzidine reagent tests.

B. <u>Blue corduroy trousers with wide brown belt</u>: The garment is heavily soiled.

Trace blood was detected.

C. White T-shirt, and D. White jockey shorts: Both garments are heavily soiled.

E. Blue necktie with "OLSEN" monogram: The tie is soiled. Trace blood was detected.

F. Beige suede cloth with pocket attached (matches Item 1): Bloodstaining, as indicated by positive benzidine reagent tests, was observed on the inner surface.

G. Black socks with white cuffs: Nothing significant was found.

H. Tan suede ankle boots: Both boots are soiled.

Item 2 - Soil from location of probable struggle and Item 3 - Soil from tire imprints: Nothing significant was found on these items.

Item 4 - Soil from location of body; Item 5 - Soil from rear tire imprints; and Item 6 - Stones: Dried blood was found in each of these items. Positive benzidine reagent and precipitin tests indicated human blood.

Item 7 - Stained paper: Positive benzidine reagent and precipitin tests indicated human blood.

A direct blood grouping test indicated group "O".

Item 8 - Decomposed blood from south side of river: Positive benzidine and precipitin tests indicated human blood.

Putrefaction preabsorbed grouping tests.

Item 9 - Cotton rope and plastic straw from river bank: Bloodstaining was observed on the rope. Positive benzidine reagent and precipitin tests indicated human blood.

Direct and absorption-elution grouping tests indicated group "B".

Item 10 - "Carta" gum and gum wrappers from left front pocket: Nothing significant was found on these items.

Item 11 - Pieces of newspaper: A tire tread impression with five 1/8" grooves 3/4" apart was observed on the paper.

CONCLUSION

Blood was found on Items 1-F, 4, 5, 6, 7, 8, and 9.

The blood on Items 4, 5, 6, 7, 8, and 9 is of human origin.

The blood on Item 7 is group "O".

The blood on Item 9 is group "B".

A tire tread impression was observed on Item 11.

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January 8, 1993

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Re: Examination of Rope and Plastic Straw
Our File No. 92-434
Report

Background

The following information was communicated to us by Michael Sullivan of the Massachusetts State Police: This anonymous case involves a homicide that occurred about 20 years ago. The names of the victim and potential suspects have not been made known to us at the present time. Pursuant to the investigation of the case a blood stained rope and a plastic straw were collected. It was requested that DNA typing be conducted on the biological evidence using the PCR DNA amplification procedure in order to determine genetic traits associated with the blood on the rope and straw.

Items of Physical Evidence

The following items of physical evidence were received from Michael Sullivan of the Massachusetts State Police on March 13, 1992 via Federal Express mail:

Items

1. Tape sealed envelope labeled "#9 next to riverbank, straw and cord" containing the following two items:

1-1. Piece of rope.

1-2. Plastic straw.

Examination of the Rope [Item 1-1].

The blood stained rope [Item 1-1] is illustrated in figure 1A. The rope was examined for the presence of blood using a sensitive presumptive test [o-tolidine and hydrogen peroxide]. Blood traces were detected along the entire length of the rope. Four areas from the rope [A, B, C and D] were removed and the DNA extracted as described below. These areas are illustrated in figures 1B and 1C.

Examination of the Partial Plastic Straw [Item 1-2].

The partial plastic straw is illustrated in figure 2A. The straw possesses a blunt end and a torn end. In addition the straw has been split down the length of the straw barrel. Presumptive tests for blood indicate that a thin film of blood is present on the straw surface down its length; and much of this thin smear is visible to the eye. Two pieces of the straw [Areas A and B] were remove from near the torn end [see figure 2B] for DNA extraction as described below.

Genetic Analysis of DNA

Recent advances in molecular biology have revealed an enormous extent of genetic variation at the level of the primary genetic material, the DNA. These findings are, to a large extent, a by-product of the recombinant DNA industry that has revolutionized the medical approach to genetic disease diagnosis and treatment. Recently it has been recognized that genetic analysis at the DNA level has particular application in the forensic sciences [Jeffreys *et al., Nature,* 316, 1985, 76-79; Gill *et al., Nature,* 318, 1985, 577-579;

Dodd, *Nature*, 318, 1985, 506-507; Jeffreys *et al.*, *Nature*, 322, 1986, 290-291; Lewin, *Science*, 233, 1986, 521-522; Tyler *et al.*, *Forens. Sci. Intern'l.*, 31, 1986, 267-272; Sensabaugh, *J. For. Sci.*, 31(2), 1986, 393-396; Kantner et al., *J. For. Sci.*, 31(2), 1986, 403-408; Giusti et al., *J. For. Sci.*, 31(2), 1986, 409-417, Higuchi *et al.*, *Nature*, 322, 1988,543-546]. Furthermore, application of DNA technology by anthropologists to mummified tissues of now extinct species is witness to the robust nature of the DNA encapsulated within the nucleus of tissue cells [Higuchi *et al.*, *Nature*, 312, 1984, 282-283; Paabo, *Nature*, 314, 1985, 644-645]. Similar recent anthropological studies have shown that the effect of profound DNA degradation is a failure to obtain any result rather than the production of a false or misleading finding [Hughes *et al.*, *Nature*, 323, 1986, 208]. The thrust of this work indicates that biological evidence is susceptible to successful analysis using DNA technology.

The San Francisco Bay area is a center for recombinant DNA research; and one of the leaders in this field is Cetus Corp. The DNA analysis in this case has been conducted employing DNA technology developed by Dr. Henry Erlich and his colleagues within the human genetics laboratory of Cetus Corp. Dr. Erlich's laboratory has been a pioneer in the study of genetic variation in the DNA associated with the HLA region of the human genome [Erlich *et al.*, *Bio/Technology*, 4, 1986, 975-981]; conventional serological HLA typing has been a routine tool for paternity testing for many years. In addition Dr. Erlich's laboratory has been involved in the development of DNA technology that is capable of amplifying relatively small quantities [sub-nanogram range] of DNA for genetic analysis [Saiki *et al.*, *Science*, 230, 1985, 1350-1354; *Saiki et al.*, *Nature*, 324, 1986, 163-166; Higuchi *et al*, *Nature*, 332,1988,543-546; Saiki *et al.*, *PNAS*, 86, 1989, 6230-6234]. The amplification strategy employed here also has been used to develop a direct test for the AIDS virus in blood [Ou *et al.*, *Science*, 239, 1988, 295-297].

The particular DNA region exploited in these studies is the DQ segment within the HLA Class II group: this region has the subclass designation DQα. The DQα DNA region can be considered a genetic marker system in its own right in a similar manner to the ABO genetic marker system. Within the DQα marker system there are 6 alleles (or traits) designated 1.1,1.2,1.3, 2, 3, and 4. Since each individual has two alleles, this

genetic marker gives rise to 21 possible types as follows: [1.1,1.1], [1.1,1.2], [1.1,1.3], etc. Each allele is associated with a specific and known DNA sequence. The DNA associated with the conventional HLA genetic markers (A, B, and C loci) is in the Class I group. All of these genetic markers are associated with the short arm of chromosome 6.

Genetic analysis of the specimens in this case involved the following essential steps:

1. Digestion of blood with SDS and proteinase K.

2. Extraction of DNA from sample digests with chloroform/phenol and concentration of DNA using Centricon molecular filters.

3. Amplification of the DQα DNA gene using the Polymerase Chain Reaction [PCR] employing 12.5 U Taq polymerase.

4. Hybridization probe analysis of the amplified sample DNA with Allele Specific Oligonucleotides (ASO's) for the six DQα alleles [1.1,1.2,1.3, 2, 3, 4] using a Dot Blot Assay.

The results of this analysis are summarized in Table 1. These findings revealed the following observed facts:

1. A low level of the DQα gene was amplified from the straw [Item 1-2] in Area A. The DQα type of this DNA was determined to be type 1.1,4. This DQα type occurs in approximately 8% of the Caucasian population and approximately 9% of the Black population.

2. The DQα gene could not be amplified or typed from the straw in Area B due to the small amount of material and inhibition by the sample of the enzyme [Taq] responsible for the amplification process.

3. The DQα gene could not be amplified or typed from any of the specimens obtained from the rope [Item 1-1] despite repeated attempts to overcome PCR inhibition.

Our File No. 92-434

Should you have any questions concerning this work, please contact us.

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