CASE REPORT

Paul Brauner,[1] *M.Sc.*

DNA Typing and Blood Transfusion

REFERENCE: Brauner P. DNA typing and blood transfusion. J Forensic Sci 1996;41(5):895–897.

ABSTRACT: As the result of a traffic accident, a man was seriously injured. Investigators found him outside the vehicle he had presumably driven. He was taken to the hospital in an unconscious state and there received a number of blood transfusions.

Bloodstains found inside the car were collected and sent for comparison with a posttransfusion blood sample of the victim (suspect). As the car involved in the accident had been stolen, the police wished to ascertain whether there was a link between the suspect and the car. No other evidence, such as fingerprints, was found in the car. Furthermore, being unconscious, the suspect was unable to give a statement.

The bloodstains from the car and the blood of the victim were tested by conventional blood group assays and DNA (RFLP and PCR). By conventional blood group assays, the effects of the blood transfusion were seen. On the other hand, the effects of the transfusion were not at all evident in the DNA assays. The implications of these results are discussed.

Transfused blood, even in large quantities, did not alter the DNA profile of the recipient in this case.

KEYWORDS: forensic science, genetic typing, blood, blood transfusion, blood typing, DNA, PCR, RFLP, D1S80

Case Report

Following a serious traffic accident, a suspect was taken to the hospital where he transfused with twelve units (300 mL/unit) of packed red blood cells. (Packed red blood cells contain leukocytes but have had plasma removed.) The same day as the transfusion, a sample of blood was taken from the suspect and sent for laboratory analysis.

From the car involved in the accident, which proved to be stolen, police removed a bloodstained piece of material from the driver's seat. Later, a second bloodstain, found on the passenger car door, was swabbed and also submitted for analysis. By comparing the bloodstains from within the car to the blood of the suspect, it was hoped to establish whether or not the suspect had driven the stolen car. (As his defense, the suspect did not deny being in the car but claimed he was a passenger rather than the driver.)

The posttransfusion blood of the suspect was compared to the bloodstains from the car by the polymorphic enzyme adenylate kinase (AK). The results obtained on the blood from the car and the suspect by AK are shown in Fig. 1. The blood from the car gave an AK 1 result, whereas the blood from the suspect gave a result similar to an AK 2-1.

The bloodstains from the car and the blood of the suspect were further analyzed by DNA. The restricted DNA of the samples (HinfI, BioLabs) and BRL ladder (DNA analysis marker system) were separated on an agarose gel and blotted onto a nylon membrane (Zeta-Probe, BioRad). The membrane was successively probed with radiolabeled D2S44(YNH24), D12S11(MS43a), D7S21(MS31), and D14S13(CMM101). The bloodstains from the car gave the same DNA profile by all four probes as the blood of the suspect. No additional bands were seen in the suspect lane by any of the four probes. The results of one probe (D2S44) are shown in Fig. 2.

One microliter of unrestricted DNA was saved for PCR amplification at the D1S80 locus (1). One tenth of a microliter of DNA was amplified (Taq polymerase, Promega) and separated on a 6% polyacrylmide gel together with an allelic ladder (Perkin-Elmer).

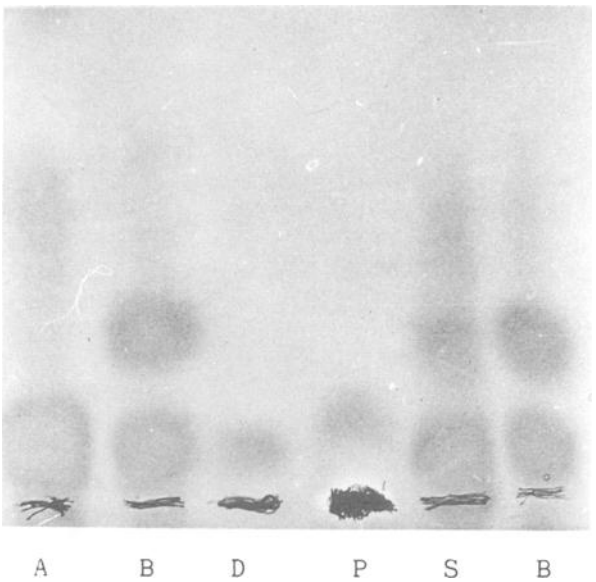

FIG. 1—*Adenylate kinase results on the blood removed from the driver's seat (Lane D), the passenger's door (Lane P), and on the posttransfusion blood of the suspect (Lane S). Lane A is an AK1 control and Lane B an AK2-1 control.*

Received for publication 4 Dec. 1995; revised manuscript received 16 Feb. 1996; accepted for publication 20 Feb. 1996.

[1]Forensic biologist, Forensic Biology Laboratory, Division of Identification and Forensic Science, Israel Police National Headquarters, Jerusalem, Israel.

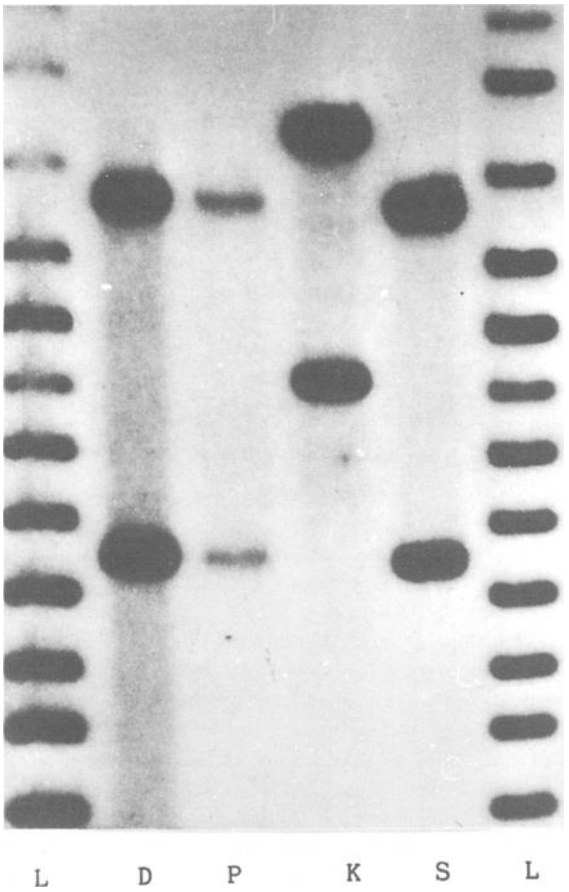

FIG. 2—*One of the single locus probe (D2S44) results on the blood from the driver's seat (Lane D), from the passenger's door (Lane P), and on the posttransfusion blood of the suspect (Lane S). Lane L is a molecular weight ladder and Lane K is the K562 control.*

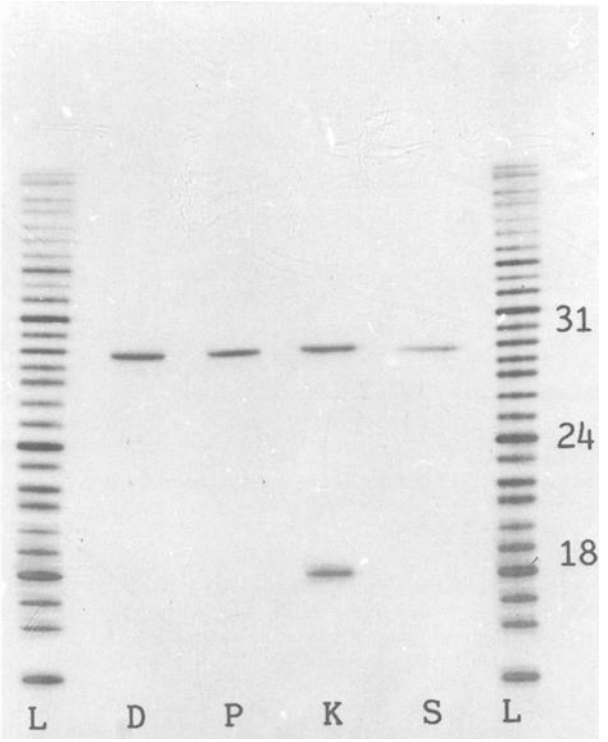

FIG. 3—*The PCR D1S80 locus results on the blood from the driver's seat (Lane D), the passenger's door (Lane P), and the posttransfusion blood of the suspect (Lane S). Lane L is the allelic ladder and Lane K is the K562 control.*

Following the separation, the bands were visualized by silver staining. A homozygotic genotype (alleles 29-29) was obtained from the bloodstains from the car as well as from the blood of the suspect (Fig. 3).

Discussion

Red blood cell "transfusion effects" have been recognized for many years (2). In the present case, this effect was observed in the polymorphic enzyme, AK, from the blood from the suspect. Two related factors may be seen as contributing to the effect. First, a significant number of units of blood were transfused into the suspect. Secondly, AK has a very low discrimination potential. Approximately 93% of the population has AK1; the remaining 7% has AK types 2-1 or 2 (3). Therefore, given the number of units of different blood with which the suspect was transfused and the low discrimination potential of AK, the probability of the suspect receiving an AK2-1 or AK2 was high. A mixture of AK1 with (transfused) AK2-1 or AK2 or both would give an electrophoretic AK2-1-like pattern. Figure 1, Lane S shows such a composite (false) AK2-1. Comparing this result to the AK from the car (Fig. 1, Lanes D and P) could have led to an erroneous exclusion of the suspect as the source of the blood. However, in a true AK2-1 (Fig. 1, Lanes B), the upper AK2 band and the lower AK1 band are of equal size. In Lane S (same figure), the unequal band size—the AK1 band being larger than the AK2 band—is indicative of a mixture of bloods. This particular difference in band sizes is consistent with an AK1 type being transfused with AK2-1/2. Such transfusion effects may be observed for a considerable period as transfused red blood cells have approximately the same lifetime as normal red blood cells (110 to 120 days) (4).

In the present case, the transfusion effect was not seen in any of the DNA assays carried out. The DNA profile of the blood from the suspect matched that of the blood from the casework items without showing any traces of additional bands. This was true in four SLP assays and even in the PCR D1S80 test which is able to detect mixtures of body fluids in which one of the fluids is present as 1/10 (0.5 ng) of the total DNA amplified (5).

Two factors would appear to be important in explaining why a transfusion effect did not occur in the DNA tests. Firstly, granulocytes, which account for approximately 70% of all white blood cells, suffer loss of function after 24 to 48 h storage at 4°C (6). More significantly, however, is the fact that only a small number of granulocytes (median 5%; range 0 to 36%) are recoverable from the circulation of a recipient after only 1 h following transfusion. Most granulocytes enter marginal or tissue pools immediately (7).

Others (8) report that transfused leucocytes are removed from a recipient's circulation within a matter of minutes. Even autotransfused leucocytes cannot be recovered after 30–90 mins.

In the normal course of forensic biology laboratory work, cases arrive in which either the suspect or the victim of a stabbing, shooting, traffic accident, or similar incident are likely to have received a blood transfusion. Since the crimes involved in such cases are likely to be of a serious nature, it may be imperative to obtain results rapidly. In our experience, with a small number of

posttransfusion cases that have reached our laboratory, DNA profiles of the recipients were not found to have been affected. With further investigation, it may be established that transfused blood has no effect on DNA profiles, thereby permitting immediate testing on such samples. (Short tandem repeat [STR] and PolyMarker [PM] loci tests on posttransfusion blood have yet to be checked for the possible presence on non-native DNA.)

References

(1) Sajantila A, Budowle B, Strom M, Johnsson V, Lukka M, Peltonen L, et al. PCR amplification of alleles at the D1S80 locus: comparison of a Finnish and a North American Caucasian population sample, and forensic casework evaluation. Amer J Hum Gen 1992; 50:816–25.

(2) Culliford BJ. The examination and typing of bloodstains in the crime laboratory, Washington, DC: U.S. Department of Justice, Law Enforcement Assistance Administration, Superintendent of Documents, U.S. Government Printing Office, Dec 1971:38–40.

(3) Stedman R. Blood group frequencies of immigrant and indigenous populations from South East England. Forensic Sci Soc 1985; 25:95–134.

(4) Mollison PL. Blood transfusion in clinical medicine, 2nd ed. Blackwell Scientific Publications, 1956:7.

(5) Cosso SI, Reynolds R. Validation of AmpliFLP™ D1S80 PCR amplification. J Forensic Sci 1995;40:424–34.

(6) Spivak JL. Fundamentals of clinical hematology. 2nd ed. Harper and Row, 1984:418.

(7) Williams WJ, Beutler E, Erslev AJ, Lichtman MA. Hematology, 3rd ed. McGraw-Hill Inc., 1977:1551.

(8) Mollison PL, IBID.,24–25.

Address requests for reprints or additional information to
Paul Brauner, M.Sc.
Forensic Biology Laboratory
Israel Police National Headquarters
Jerusalem, Israel 91906