PERSISTENCE OF GM ALLOTYPES IN BLOODSTAINS EXPOSED TO ADVERSE CONDITIONS

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INTRODUCTION

The inherited structural differences in the heavy chains of IgG, known as GM allotypes, can be exploited serologically for the partial genetic characterization of bloodstain evidence [Ducos et al 1963; Khalap et al 1976; Kipps 1979]. The favorable distribution of phenotypes in United States populations [Schanfield 1987], and the long term stability of these antigens in dried bloodstains [Hoste et al 1978] were factors that encouraged an evaluation of the GM allotyping methodology for potential use in the FBI Laboratory.

This report is a portion of that evaluation and describes the results of our assessment of the detectability of GM allotypes in bloodstains contaminated by various substances and stored under conditions potentially deleterious to proteins.

MATERIALS AND METHODS

Allotyping procedure

Bloodstain extracts were tested for GM A,X,F.B0,C3C5,G,G5 by the method of Schanfield [1987]. All tests were performed in V-bottom microplates by the sequential addition of 25 uL bloodstain extract, 25 uL anti-allotype antiserum, and 25 uL anti-D coated group O R_1R_1 erythrocytes. The plates were incubated at 4^0C for one hour followed by centrifugation at 1000 RCF for one minute. The test results were read macroscopically 10-20 minutes after tilting the plates at an angle of 60^0. Positive and negative control samples were run simultaneously on each microplate. All allotyping reagents were obtained from Allotype Genetic Testing, Atlanta, GA.

Bloodstain preparation and storage

Clean, well-worn denim blue jeans served as the fabric substrate for the preparation of bloodstains. Defined areas on both legs of the jeans were treated with each of the following substances: gasoline, lubricating grease, brake fluid, unused motor oil, used motor oil and ground-in red clay (dirt). Within two hours of the application of these substances, multiple 25 uL aliquots of fresh whole blood (GM phenotype: A,X,F.B0,G,G5) were pipetted onto each of the contaminated areas of the jeans. In other areas, blood deposition was followed immediately by the addition to the stains of either semen or urine. Bloodstains were placed also in in uncontaminated regions of the fabric. The stains were allowed to dry overnight at room temperature. The following morning, the jeans were split to obtain duplicate stain panels. One panel was maintained in the laboratory at room

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temperature for the duration of the study period. The other stain panel was covered with clear plastic wrap and placed on the roof of the laboratory building facing due south. At weekly intervals, replicate bloodstains were removed from each test and control area of the indoor and outdoor arrays for determination of the GM allotypes present. Stains were extracted for one hour at room temperature in one ml of PBS containing 1% bovine serum albumin. The concentration of albumin in the bloodstain extracts was determined by enzyme-linked immunosorbent assay [unpublished observations].

RESULTS AND DISCUSSION

The detectability of allotypes in blood deposited on blue jeans that were maintained in the laboratory for the duration of the study period (Table 1), was affected only by the presence of urine. By the fourth week after preparation, two thirds of the allotypes were undetectable in bloodstains mixed with urine; by the sixth week, none could be detected.

A quite different picture of allotype detectability was seen with the identical array of bloodstains that had been maintained outdoors (Table 2). After only one week, incomplete phenotypes were obtained for blood that had been mixed with used motor oil, brake fluid, semen, and urine. The ensuing weeks of storage rendered the allotypes in other bloodstain/contaminant mixtures undetectable. By the sixth week of study, the only allotypes detectable were X and F in blood contaminated by clean motor oil.

In a companion series of studies, it was noted that the recoverability of albumin from extracts of these bloodstains appeared generally to diminish in parallel with the inability to detect the allotypes (Table 3). More albumin was recoverable from the bloodstains kept indoors, and these stains also yielded the greatest number of extracts with complete GM phenotypes.

These studies indicate that some substances that can commonly contaminate evidentiary materials are not likely to affect the detection of the GM allotypes as long as the evidence has been maintained under reasonable conditions of temperature and humidity. The outdoor exposure of bloodstains to direct sunlight for as little as one week in the presence of several contaminants (e.g. used oil, semen or urine) can be expected to result in a loss of allotype detectability.

The inability to extract albumin from the outdoor stains suggested to us that the allotypes might not have been destroyed by the adverse conditions, but had become undetectable because of resistance of the bloodstain immunoglobulins to extraction. This belief was supported by the observation that detectability of some of the allotypes could be restored 30 weeks after outdoor storage if the extractions were carried out for 14 days (data not shown).

The present study corroborates the observations of others, that the GM allotypes present in dried bloodstains are robust. GM allotypes were readily demonstrable in dried stains kept at room temperature for at least six weeks despite contamination by adventitious substances. Within this category of stains, only urine appeared to have a deleterious influence on allotype detectability. Although extremes of temperature and humidity, in concert with some contaminants, appeared to compromise the detectability of GM allotypes in dried stains, much of this behavior could be attributed to a probable inability to readily solubilize the immunoglobulins rather than actual loss of antigenicity.

Table 1. Detectability of GM allotypes in dried bloodstains[1] contaminated by various substances and stored indoors at ambient temperature.

	Allotypes absent at week:				
Contaminant	1	2	3	4	6
None	N[2]	N	N	N	N
Gasoline	N	N	N	N	N
Grease	N	N	N	N	N
Clean Oil	N	N	N	N	N
Used Oil	N	N	N	N	N
Brake fluid	N	N	N	N	N
Dirt	N	N	N	N	N
Semen	N	N	N	N	N
Urine	N	N	N	A.B0	A,X,F.B0,G,G5

[1] Bloodstains prepared with blood of phenotype GM: A,X,F.B0,G,G5
[2] N = None absent

Table 2. Detectability of GM allotypes in dried bloodstains[1] contaminated by various substances and stored outdoors.

	Allotypes present at week:				
Contaminant	1	2	3	4	6
None	A,X,F.B0,G,G5	A,X,F.G.G5	X,F	X	N[2]
Gasoline	A,X,F.B0.G.G5	A,X,F	N	N	N
Grease	A,X,F.BO.G.G5	A,X,F.G.G5	N	N	N
Clean oil	A,X,F.B0,G,G5	A,X,F.G	A,X,F.G	A,X,F.G	X,F
Used oil	A,X,F.G5	A,X,F.G5	N	N	N
Brake fluid	X.G5	N	A,X,F.B0,G5	X,F	N
Dirt	A,X,F.B0.G,G5	ND[3]	A,X,F.B0,G5	X,F	N
Semen	X.G5	N	G5	N	N
Urine	G5	N	N	N	N

[1] Bloodstains prepared with blood of phenotype GM: A,X,F.B0,G,G5
[2] N = None detectable
[3] ND = Not done

Table 3. Detectability of GM allotypes and recovery of serum albumin from dried bloodstains

	PERCENT RECOVERY OF ALBUMIN							
	OUTSIDE					INSIDE		
Stain Contaminant	1	2	3	4 -weeks-	1	2	3	4
NONE	24	15	8	8	-	97	45	33
GASOLINE	14	13	6	8	-	80	47	37
GREASE	42	43	9	11	-	95	51	40
NEW OIL	30	54	30	19	-	95	51	40
USED OIL	ND	38	6	11	-	91	49	28
BRAKE FLUID	4	10	10	12	-	89	50	34
SEMEN	6	8	5	6	-	87	50	41
URINE	3	6	4	6	-	52	27	7

00 = Complete GM phenotype detected
00 = Partial phenotype/ no allotypes detected

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