TECHNICAL NOTE

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Immunofixation of Complement Component C3 Phenotypes in Bloodstains After Cellulose Acetate Electrophoresis

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ABSTRACT: The determination of the polymorphic C3 phenotypes was accomplished by electrophoresis on cellulose acetate followed by immunofixation. The three common phenotypes resulting from the two common codominant alleles $C3^S$ and $C3^F$ were clearly distinguishable in blood and bloodstain samples. Storage and degradation of C3 in blood samples as well as the stability of C3 in dried bloodstains is discussed.

KEYWORDS: forensic science, genetic typing, immunofixation, electrophoresis, complement C3

Complement C3 plays a key role in both the classic and alternate pathways of complement activation. Perhaps because of this key role C3 is found in human serum at concentrations (1500 μg/mL) greater than any of the other complement components. The biological activity of Complement C3 includes anaphylatoxin, chemotaxis, immune adherence (opsonization), and lymphocyte activation. Complement C3 is produced in the liver and has a molecular weight of approximately 180 000 daltons. C3 is made up of two nonidentical polypeptide chains which are held together by disulfide bonds and by noncovalent interactions. C3 is highly labile and is converted into a number of conversion products, the major one being C3c also known as Pt or post transferrin [*1,2*]. The C3c fragments have been reported to correspond with the C3 phenotype [*2*].

Wieme and Demeulenaere [*3*] described the genetically determined electrophoretic variant of human Complement C3. C3 is of autosomal codominant inheritance, exhibiting three common types and at least 22 variants and a silent allele [*2,4-6*]. These alleles reside on Chromosome 19 [*5*].

The majority of the literature describes the electrophoretic techniques for C3 as extended agarose or starch gel electrophoresis, with general protein staining [*6,7*].

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In 1982 Gremenis et al [*8*] described a technique in which the electrophoresis was performed on Cellogel with visualization by immunofixation with specific C3 antiserum. The purpose of this experiment was threefold: (1) adapt the Cellogel procedure to Sartorius cellulose acetate membranes which are readily available in the United States, (2) test the stability of Complement C3 in dried bloodstains, and (3) access the application of this system for forensic science work.

Experimental Procedure

The electrophoresis was performed on both the Sartophor system and the Beckman microzone using sartorious cellulose diacetate membranes (Sartorius Filters, Inc. Hayward, CA; 12200-70-145BN and 12200-57-145BN).

The tank and membrane buffers were 0.037*M* Tris(hydroxymethyl)aminomethane (4.5 g) and 0.290*M* glycine (21.8 g) made up to 1 L with deionized (DI) water, pH 8.4 (for the Sartophor the membrane buffer was diluted 1:2).

Fresh serum samples were diluted 1:2 with deionized water. Bloodstains (5 mm^2) were extracted in approximately 30 μL of DI water followed by 30 s of vortexing in three volumes of chloroform.

The cellulose acetate membrane was soaked with the tank buffer, blotted, and placed on the tank bridge. The bridge was then placed into the electrophoresis tank. A single application at the most cathodic position was required for serum samples, while four to six applications were sometimes necessary for bloodstains. Electrophoresis was performed at 500 V for 70 min, at room temperature.

Upon completion of electrophoresis, the membrane was placed face down in a 1:3 dilution of anti-human C3 (Sigma C-7761) for 10 min. No significant lot-to-lot variations were observed in the antisera. The membrane was washed $\times 2$ with cold saline for 5 min each, and then the membrane was stained for 10 min in Crowle's Double Stain [Crowle's Double Stain solution consists of 2.5-g crocein scarlet 7B (Sigma C-3643), 150-mg coomassie brilliant blue R-250 (Sigma B-0630), 50-mL glacial acetic acid, and 30-g trichloroacetic acid diluted to 1L with deionized water]. Excess stain was removed with 3% acetic acid until adequate contrast between the background and the C3 bands were obtained. The membrane was then dried between blotters and labeled to become a permanent record.

Results and Discussion

Figures 1 and 2 illustrate the three common genetic phenotypes of C3. F is the fastest and S the slowest migrating band on electrophoresis. Type FS contains both bands. A weakly stained band, migrating anodally to the main band, is observed in every sample.

Figure 3 shows an unusual pattern with a band migrating cathodal to the S band. The pattern was observed in two fresh serum samples. Unfortunately we were unable to do family studies and there was an insufficient amount of sample for submission to the C3 reference laboratory in Germany.

Bloodstains were prepared by drying whole blood on glass and cotton and then stored at room temperature. C3 phenotypes could be determined on cotton up to one week of age and on glass up to two weeks.

Twenty samples were phenotyped by the above describe procedure and by high resolution agarose gel electrophoresis [*7*] with complete correlation.

In a study of 393 unrelated whites the gene frequencies for C3 were; $C3^S$ 0.780, $C3^F$ 0.218, and $C3^{variant}$ 0.002. The distribution of the C3 phenotypes is shown in Table 1. A limited study of 31 blacks resulted in gene frequencies of: $C3^S$ 0.952 and $C3^F$ 0.048. A second set of black samples were obtained from a local hospital. These samples were from patients. Hospital policy prevented the disclosure of the patients' names and illnesses. For this reason the related-

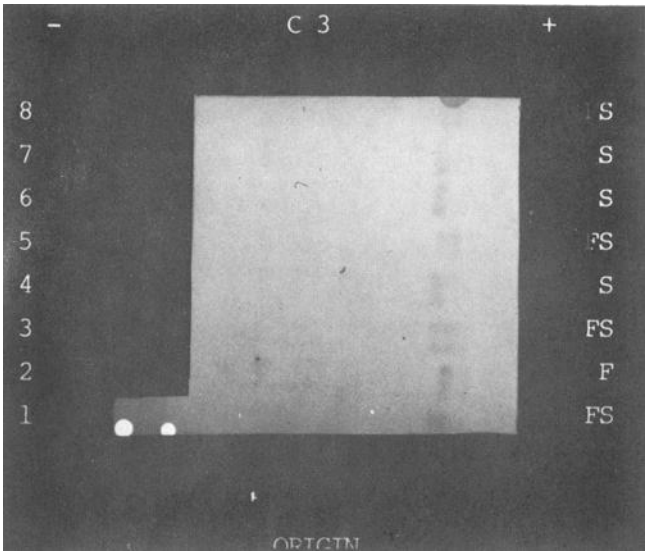

FIG. 1—*Three common phenotypes of C3.*

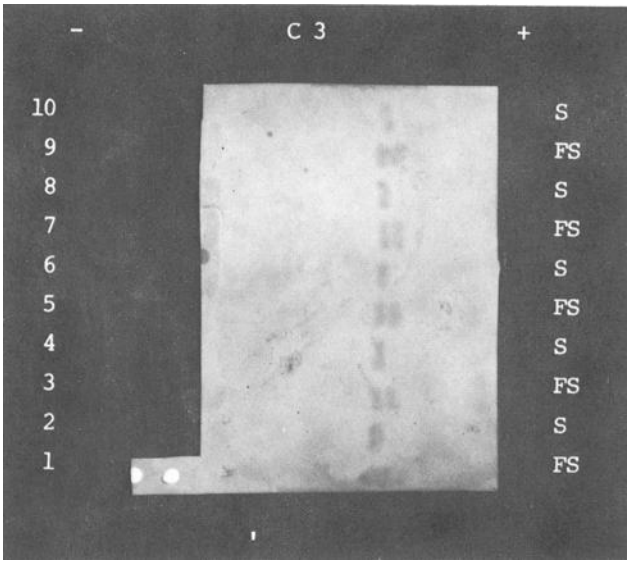

FIG. 2—*Two common phenotypes of C3 using the Sartophor system.*

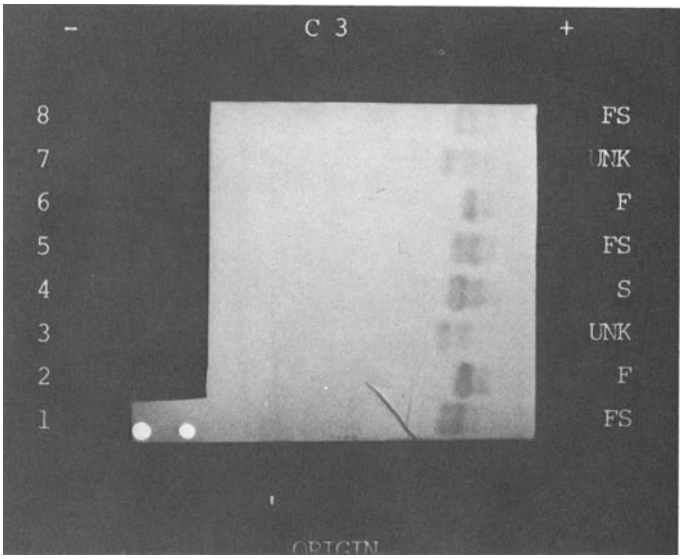

FIG. 3—*Unknown variant in the C3 system.*

TABLE 1—*Distribution of the C3 phenotypes in the white samples.*

Phenotype	Expected Frequency	Expected Number	Observed Number
S	0.61	239	232
FS	0.34	134	149
F	0.05	20	11
Total	1.00	393	392[a]

[a]One rare variant was observed.

TABLE 2—*Distribution of the C3 phenotypes in the black samples.*

Phenotype	Expected Frequency	Expected Number	Observed Number
	LAB SAMPLE		
S	0.906	28.1	29
FS	0.091	2.8	1
F	0.002	0.1	1
Total	0.999	31.0	31
	HOSPITAL SAMPLE		
S	0.92	369	379
FS	0.08	32	22
F	0.002	0.8	4
Total	1.002	401.8	401

ness of the patients and the real possibility of multiple samples from a single patient prevent the data being used for population studies and is only presented here for informational purposes. Five hundred and forty eight samples were collected and tested. One hundred and forty seven of the samples failed to yield visible C3 banding patterns in our system. Reduced levels of C3 were expected in some of the hospital samples because of C3's role in the immune response [*1*]. The remaining 401 samples had gene frequencies of $C3^S$ 0.96 and $C3^F$ 0.04. The distribution of the C3 phenotypes in the two black samples are shown in Table 2. No deviation from Hardy-Weinberg equilibrium is indicated ($p = 0.05$, 1 d.f.). The distribution of the three common phenotypes agree with previously published data [*2,4,6,7,9*].

Conclusion

The procedure described works well with fresh serum samples and fresh dried bloodstsins stored under optimum conditions. However the low stability of the C3 protein limits its applications in forensic serology. Experimentation is currently being conducted to see if the more stable degradation fragment C3c can be consistently phenotyped in older bloodstains.

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