Crossed Electroimmunodiffusion and Bloodstain Investigations

The use of crossed electroimmunodiffusion (CEID) for the examination of bloodstains as described by Sweet and Elvins (*1*) has been the subject of a number of investigations (*2*) since publication of the early report by Whitehead *et al.* (*3*). Work at the Home Office Central Research Establishment and Loughborough University of Technology in England has been concerned with examining some of the difficulties to which Sweet and Elvins refer in their interesting report.

We believe the major problems to be considered are:

1) What degree of discrimination can be achieved from the CEID profile when a very large population is studied, as distinct from the ten individuals studied by Sweet and Elvins (*1*)?

2) Which proteins are sufficiently stable to retain both their antigenic activity and solubility characteristics (which may not be the same thing) under the many varied conditions that bloodstains present themselves in the course of crime detection?

3) What advantages has CEID serum protein profiling, which is based on a study of essentially continuous variables, over the traditional methods based on discrete genetically determined factors?

The degree to which the concentration of a protein in blood will "discriminate" between individuals (the word "discriminate" is to be preferred over "individualize" since the latter has yet to be proved of CEID) can best be approached by using the statistical techniques developed by Jones (*4*) and further extended by King (*5*) for considering the discrimination achieved when studying continuous variables in blood. The "discriminating power" (DP) of a given variable, for example, the concentration of a given protein, is defined as

$$DP \simeq 1 - 2.58/\pi^{1/2}\, Sp/Sg$$

where Sp is the average personal (intraindividual) standard deviation and Sg the population (interindividual) standard deviation.

It follows that a satisfactory appraisal of the role of individual proteins in a profiling system cannot be achieved without adequate information concerning personal (Sp) and population (Sg) variations in concentration of a given protein. Although such data could be obtained by studying a sufficiently large population of "normal" healthy individuals, further problems may arise when considering the "acute phase reactants," a group of proteins in serum whose concentration increases markedly as a result of illness, or even stress (*6*).

The difficulties mentioned above relate only to attempts to discriminate between *blood*. Any attempt to apply CEID to bloodstains in a similar manner introduces new problems of both antigenic stability and quantitative extraction of proteins from stains. Efforts, therefore, to relate blood from a living or dead person to bloodstains produced during a previous "incident" must take into account all of the above considerations.

Finally, what advantages may be expected from such a system over traditional grouping techniques based on genetically determined markers? Recent years have seen considerable advances in the use of polymorphic protein systems, including enzymes for typing bloodstains, in addition to the traditional serological markers such as ABO (*7*). In the Metropolitan Police Forensic Science Laboratory, London, 13 grouping systems are in routine use for typing bloodstains. These include ABO, rhesus, phosphoglucomutase, haptoglobin, erythrocyte acid phosphatase, adenylate kinase, adenosine deaminase, and more recently the Gm and Km systems. Clearly, a combination of particular groups in a bloodstain may lead to very high levels of discrimination indeed, depending on the frequency of the phenotypes (*8*).

The main value of CEID may be in obtaining information not normally available from a study of the blood genetic markers listed above. As Sweet and Elvins point out (*1*), some indication of sex may be obtainable. In any event I believe that very much more work is required before the results of CEID investigations on bloodstains become reliable enough for court production.

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References and Notes

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We welcome Whitehead's comments on some of the problems in using crossed electroimmunodiffusion (CEID) to examine bloodstains and his mention of the advantages of traditional techniques. At the time of our investigation we were aware of those problems and advantages although they were not extensively discussed in the report (*1*). Some were considered more fully in our subsequent publication (*2*).

Although Whitehead's only explicit criticism of our report, with which we do not take issue, was the use of the word "individualize" instead of "discriminate," we consider it important to emphasize some novel features of the investigation which may be obscured by the wording of his comments.

To our knowledge, the work was the first to provide published (*1, 2*) "hard" data on the important question of intraindividual versus interindividual variations in the amounts of bloodstain antigens, as revealed by CEID, and to show that the variations were such as to permit discrimination among the subjects in the study.

In general, we do not object to the use of statistical techniques, as proposed by Whitehead, in considering the discriminatory power of continuous variables in blood. However, in our study, simply ascribing significance only to those CEID peaks whose ranges in heights were completely different for any two individuals being compared was a more stringent, and therefore more desirable, criterion for discrimination.

Finally, we agree with Whitehead that much more work is required before CEID results are reliable enough for court production. We hope that the appearance in *Science* of Whitehead's comments and our report will help stimulate a broader interest in exploring the potential of CEID and related techniques in forensic serology.

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