donate and the ADP that it releases from the platelets; CP/CPK inhibits only the contribution made by ADP. The inhibitory effect of CP/CPK on the release of [^{14}C]serotonin from the previously labeled platelets is probably related to its inhibition of the synergistic effect of ADP on the release reaction (*11*).

Thrombin-degranulated platelets have little if any releasable ADP, and CP/CPK has no effect on their shape change or aggregation induced by collagen or the ionophore A23,187 (*8*). In contrast, with intact platelets, CP/CPK inhibits aggregation induced by the ADP released by collagen, thrombin (*11*), or sodium arachidonate. Our observations show that sodium arachidonate can induce shape change and aggregation of platelets that no longer have releasable constituents in their amine storage granules. Thus sodium arachidonate can cause aggregation that is not mediated by released ADP. It appears that sodium arachidonate has effects that are somewhat similar to the action of ADP and the ionophore A23,187 in that sodium arachidonate can induce shape change and aggregation of platelets whether or not ADP is released from the platelet granules.

However, the mechanisms by which ADP and the ionophore A23,187 induce shape change and aggregation of thrombin-degranulated platelets appear to be different from that involving sodium arachidonate since indomethacin and ASA inhibit the sodium arachidonate–induced platelet changes but do not inhibit aggregation induced by ADP or the ionophore A23,187 (*8*, *12*).

Platelet aggregation induced by sodium arachidonate has been attributed to the formation of the endoperoxide PGG_2 (*5*) or thromboxane A_2 (*3*); evidence has been presented that the cyclooxygenase (*13*) responsible for the formation of PGG_2 is inhibited by ASA and indomethacin (*5*). Roth and Majerus (*14*) have shown that ASA acetylates cyclooxygenase; this may be responsible for the inhibitory effects of ASA on the formation of PGG_2 and the thromboxanes. Sodium arachidonate–induced aggregation of thrombin-degranulated platelets is probably due to endoperoxides or thromboxanes.

R. L. KINLOUGH-RATHBONE
H. J. REIMERS
J. F. MUSTARD
Department of Pathology,
McMaster University,
Hamilton, Ontario L8S 4J9, Canada
M. A. PACKHAM
Department of Biochemistry,
University of Toronto,
Toronto, Ontario M5S 1A1, Canada

References

1. M. J. Silver, J. B. Smith, C. Ingerman, J. J. Kocsis, *Prostaglandins* **4**, 863 (1973); B. B. Vargaftig and P. Zirinis, *Nature (London) New Biol.* **244**, 114 (1973).
2. J. B. Smith, C. Ingerman, J. J. Kocsis, M. J. Silver, *J. Clin. Invest.* **53**, 1468 (1974).
3. M. Hamberg, J. Svensson, B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2994 (1975).
4. A. L. Willis and D. C. Kuhn, *Prostaglandins* **4**, 127 (1973); B. B. Vargaftig, *Agents Actions* **3**, 357 (1973).
5. C. Malmsten, M. Hamberg, J. Svensson, B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1446 (1975).
6. N. G. Ardlie, D. W. Perry, M. A. Packham, J. F. Mustard, *Proc. Soc. Exp. Biol. Med.* **136**, 1021 (1971).
7. J. F. Mustard, D. W. Perry, N. G. Ardie, M. A. Packham, *Br. J. Haematol.* **22**, 193 (1972).
8. R. L. Kinlough-Rathbone, A. Chahil, M. A. Packham, H.-J. Reimers, J. F. Mustard, *Thromb. Res.* **7**, 435 (1975).
9. H.-J. Reimers, M. A. Packham, R. L. Kinlough-Rathbone, J. F. Mustard, *Br. J. Haematol.* **25**, 675 (1973).
10. R. L. Kinlough-Rathbone, M. A. Packham, J. F. Mustard, *ibid.* **19**, 559 (1970).
11. M. A. Packham, M. A. Guccione, P.-L. Chang, J. F. Mustard, *Am. J. Physiol.* **225**, 38 (1973).
12. J. F. Mustard, D. W. Perry, R. L. Kinlough-Rathbone, M. A. Packham, *ibid.* **228**, 1757 (1975).
13. M. Hamberg and B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3400 (1974).
14. G. J. Roth and P. W. Majerus, *J. Clin. Invest.* **56**, 624 (1975).

31 October 1975; revised 17 February 1976

Human Bloodstains: Individualization by Crossed Electroimmunodiffusion

Abstract. *Crossed electroimmunodiffusion was evaluated as a means for establishing the individuality ("fingerprinting") of human bloodstains. In ten separate examinations on stains from each of ten persons there was at least one peak with a unique range in height so that individualization was possible. The heights of certain peaks showed statistically significant female-male differences.*

Individualization or "fingerprinting" of human bloodstains is probably the primary unrealized goal of forensic serology. Improved and extended typing of erythrocytes (*1*) can approach this goal but is inherently incapable of reaching it because no human is unique in any one or combination of the known types.

We reasoned that if serologic individualization is possible it will come from use of a technique that goes beyond the qualitative distinctions made by typing and includes as well a quantitative aspect. Since crossed electroimmunodiffusion (CEID) is among the most sensitive techniques with these characteristics, we chose to evaluate it as a means for individualization. An earlier preliminary report by Whitehead *et al.* (*2*) established that eluted bloodstain antigens are reactive and apparently distinguishable by CEID.

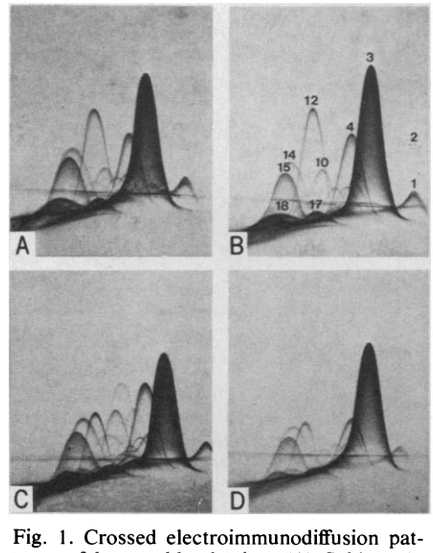

Fig. 1. Crossed electroimmunodiffusion patterns of human bloodstains. (A) Subject A, day 24, (B) Subject A, day 41. Those peaks identified as particular proteins are: 1, α_1-lipoprotein; 2, prealbumin; 3, albumin; 4, α_1-antitrypsin; 5, Gc globulin; 10, ceruloplasmin; 12, haptoglobin; 14, hemopexin; 15, transferrin; 17, α_2-macroglobulin; and 18, immunoglobulin A. (C) Subject B, day 57. (D) Subject G, day 48.

Blood for stains was obtained by finger puncture on ten different occasions over a 4-month period from five female and five male college students, all apparently healthy and ranging in age from 22 to 35 years. Blood was collected on pieces of a cotton bed sheet, allowed to dry for 48 hours, and then eluted by placing sections (6 mm in diameter) of the bloodstained sheet into a 2-ml beaker containing 0.05 ml of 0.85 percent NaCl, buffered to *p*H 7.2 with 0.02*M* sodium phosphate (PBS). After 5 minutes blood was withdrawn and diluted with PBS to a concentration of 10 mg of protein per milliliter (*3*). Antiserum to whole human serum was prepared in each of two rabbits by making 21 subcutaneous injections of 0.3 ml of serum in 0.3 ml of complete Freund's adjuvant over a 14-week period. Rabbits were then exsanguinated; the serums were pooled, dispensed into 7-ml portions, and stored at −20°C. The CEID was conducted on microscope slides (5 by 8 cm) as previously described (*4*). Initial experiments, in which antigen (bloodstain) and antibody were varied, led to the choice of 0.01 ml of antigen and 0.3 ml of antibody as optimum for routine assay. With these conditions as many as 22 antigens, some of which

were identified as particular serum proteins by use of monospecific antiserums (Behring Diagnostics), could be resolved as separate precipitin peaks (5).

In Fig. 1 CEID patterns are shown of stains from the same individual obtained on two occasions and patterns from two other individuals obtained on single occasions. The close similarity of patterns from the same subject (Fig. 1, A and B) and the gross differences among subject patterns (Fig. 1, compare A and B with C and D) indicate that CEID is potentially able to individualize a stain. However, the demonstration of that potential requires evidence that any variations in the number and height of precipitin peaks in assays of an individual's blood are distinguishable from differences among individuals. Ordinarily, acceptable evidence is in the form of probability statements from statistical significance calculations. For this study we deemed it appropriate to ascribe significance or value to a precipitin peak only if its range in height, over several determinations, was completely different for any two individuals being compared. Table 1, which is divided into quadrants to facilitate female-female, male-male, and female-male comparisons, shows the number of such peaks with nonoverlapping ranges in each of the 65 comparisons between subjects. The fact that a number larger than 0 appears in all comparisons signifies that each of the ten subjects could be distinguished from all others by the criterion mentioned above. Whether the same would hold true for the general population is, of course, open to question. However, we emphasize that (i) our criterion for individuality was more stringent than is usual and (ii) the group of subjects was relatively homogeneous in age, race, and state of health, both of which argue for a greater overall discriminatory power of CEID than we observed.

The only relatives in the study were subjects I and J, who are brothers. Although they showed significant difference in only one peak, that difference was no less than in several other comparisons. Further, the only subject of non-European extraction (D, Persian) was no more easily distinguishable than most other subjects. These results would not support a contention that CEID patterns reflect phenotypic characters similar to those detected in traditional erythrocyte typing. By determining the average value for entries in each quadrant, one can estimate the relative degrees of differences among females, among males, and between females and males. When this was done it was found, as expected, that the greatest difference was between females and males (average of 4.1). What was not necessarily expected was a considerably greater difference among females (average of 2.9) than among males (average of 1.9). This suggests that in forensic practice what is probably the most commonly desired distinction, male from male, will perhaps be the most difficult to make. On the other hand, there is a positive aspect to the dependence of individualization on differences in a small number of antigens. Clearly, if the antigens could be identified and those showing no significant interindividual differences eliminated from the CEID pattern (by use of only those antibodies with specificities for significant antigens), the analysis and interpretation of results, which is always a problem in procedures such as CEID, could be greatly simplified.

Table 1. Distinction between subjects on the basis of nonoverlap in ranges of heights of CEID peaks.

Subject	Female					Male				
	A	B	C	D	E	F	G	H	I	J
Female										
A		3*	2	3	4	3	4	2	1	4
B	3		1	7	2	5	5	4	5	9
C	2	1		4	1	3	4	2	4	8
D	3	7	4		5	4	3	3	2	4
E	4	2	1	5		6	4	7	4	9
Male										
F	3	5	3	4	6		2	2	2	4
G	4	5	4	3	4	2		2	1	2
H	2	4	2	3	7	2	2		1	2
I	1	5	4	2	4	2	1	1		1
J	4	9	8	4	9	4	2	2	1	

*Number of peaks with nonoverlapping ranges in subject-subject comparison.

To gain at least a preliminary indication of which antigens are of significance, we (i) determined the number of times each of the 22 precipitin peaks was of value (nonoverlapping ranges) in the 65 comparisons between subjects, the 20 female-female comparisons, and the 20 male-male comparisons and (ii) used monospecific antiserums (6) to identify as particular proteins as many as possible of the 22 antigens. The results, shown in Fig. 2, were somewhat surprising and carry important implications. Whereas 15 of the 22 antigens were of value in more than one overall comparison, only nine and five antigens, respectively, were of any value in female-female and male-male comparisons. Moreover, those antigens of value in female-female distinctions were largely different from those of value in male-male distinctions. Thus, patterns could be simplified by use of only significant antibodies, but the nature and extent of the simplification would depend on prior knowledge or judgment of the sex of the person from whom the blood came.

We were successful in identifying as particular proteins 11 of the 22 antigens (Fig. 1B). Four of them (albumin, Gc globulin, α_2-macroglobulin, and immunoglobulin A) had overlapping ranges in all comparisons and thus were of no value in individualization. Similarly, in female-female comparisons four peaks (α_1-lipoprotein, prealbumin, haptoglobin, and hemopexin) and in male-male comparisons five peaks (α_1-lipoprotein, α_1-antitrypsin, ceruloplasmin, hemopexin, and transferrin) were of no value. Unfortunately we could not identify peaks 8, 11, and 20, three of the most valuable overall and intrasexual distinguishing antigens (Fig. 2).

The success of CEID in forensics depends, of course, on retention of activity

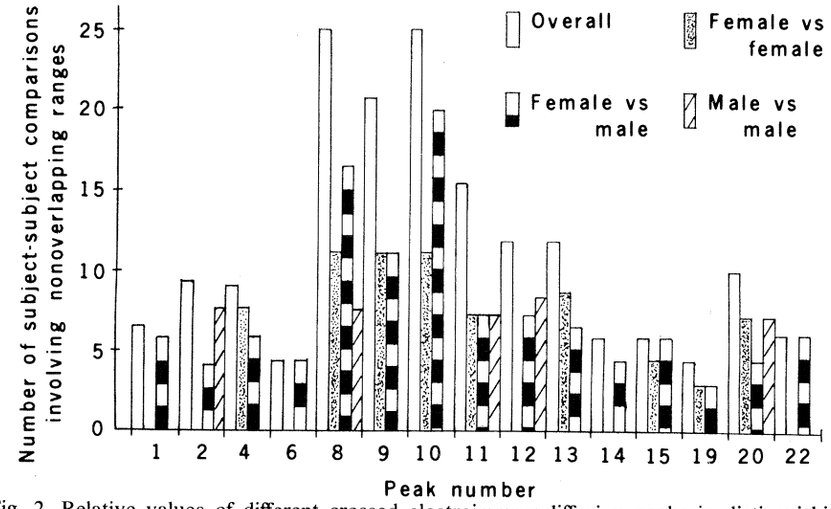

Fig. 2. Relative values of different crossed electroimmunodiffusion peaks in distinguishing females from females, males from males, females from males, and overall. Those peaks of value in one or no distinctions are not shown.

of bloodstain antigens for a period of time following deposition. Thus, we routinely allowed stains to stand for 48 hours before analysis on the reasonable assumption that most crimes of violence are discovered and reported within that period. Nevertheless, it was of interest to obtain some tentative indication of antigenic stability over longer periods. Accordingly, stains from three subjects (A, E, and G) were allowed to stand for 16 days and analyzed as above. The major findings were that as many as 5 of the 22 antigens were not resolved but individualization was still possible. In fact, in all three comparisons the number of peaks with nonoverlapping ranges was equal to or greater than the number in comparisons of 48-hour stains.

Aside from our evaluation of CEID as a means for individualization, we considered it of interest to examine the value of the technique to distinguish female from male bloodstains because the best available procedure, Y chromosome fluorescence, is reliable in only about 50 percent of the cases (*7*). We found (*8*), as did Clarke and Freeman in studies of fresh serum (*9*), significant (Student's *t*-test) sexual differences in the amounts of several antigens, most notably α_1-lipoprotein ($P < .02$) and ceruloplasmin ($P < .01$). These results suggest a serious consideration of CEID as a way to judge the probability of a stain having come from one sex or the other.

GEORGE H. SWEET
Department of Biological Sciences, Wichita State University, Wichita, Kansas 67208

JAMES W. ELVINS
Department of Administration of Justice, Wichita State University

References and Notes

1. S. Kind, *Nature (London)* **185**, 397 (1960); A. Fiori, M. Marigo, P. Benciolini, *J. Forensic Sci.* **8**, 419 (1963); L. Nickolls and M. Pereira, *Med. Sci. Law* **2**, 172 (1962).
2. P. Whitehead, S. Kind, P. Morris, M. Davies, R. Cleevley, *J. Forensic Sci. Soc.* **10**, 83 (1970).
3. O. Warburg and W. Christian, *Biochem. Z.* **310**, 384 (1941).
4. G. Sweet *et al.*, *J. Immunol.* **111**, 554 (1973).
5. Larger amounts of antigen or antibody, or of both, gave more precipitin peaks, but many of them were superimposed; others had no discernible apex, and thus their heights could not be measured, and the overall pattern was more difficult to interpret. The slide of subject A for day 41 (Fig. 1B) served as the master pattern for locating and numbering the corresponding peaks in all other slides.
6. B. Weeke, *Scand. J. Clin. Lab. Invest.* **25**, 269 (1970).
7. A. Phillips and D. Webster, *J. Forensic Sci. Soc.* **12**, 361 (1972).
8. G. H. Sweet and J. W. Elvins, *J. Forensic Sci.*, in press.
9. H. Clarke and T. Freeman, *Clin. Sci.* **35**, 403 (1968).
10. We thank Dr. E. Bubieniec for reviewing the manuscript, and R. Cimprich, F. Eftekhar, S. Elvins, D. Krkosska, R. Pettriess, F. Riffel, M. Schroeder, P. Schroeder, and D. Williamson for serving as blood donors.

8 March 1976

Human Pancreatic Islets in Culture: Effects of Supplementing the Medium with Homologous and Heterologous Serum

Abstract. *Islets of Langerhans, isolated from the human fetal pancreas and cultured in media supplemented with serums from human umbilical cord or adult donors, remained intact, free-floating, and functional for 2 to 5 months. Substitution of fetal calf serum for the human serum usually resulted in attachment of islets, monolayer formation, and relatively early (3 weeks) functional arrest.*

The ability to isolate pancreatic islets of Langerhans and maintain them in culture in a functional state for extended periods of time should be useful for the investigation of their normal and abnormal physiology. It would provide tissue for eventual attempts at transplantation. Here we report a technique that has allowed intact islets from the human fetal pancreas to remain functional for as long as 150 days and, by varying the condition of the culture medium, to produce functional monolayers of islet cells.

There are many methods for the isolation of islets and the preparation of functional monolayers of endocrine cells of the pancreas of rat, mouse, and guinea pig, but the monolayers either undergo

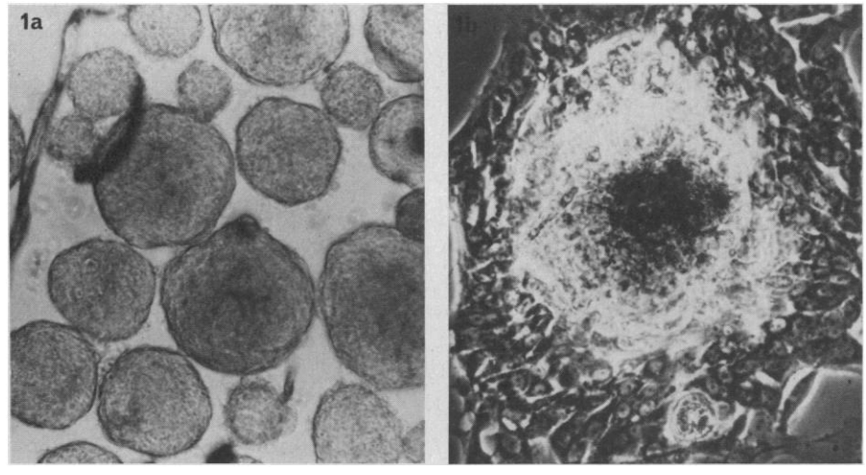

Fig. 1. (a) Intermediate-power phase micrograph of a cluster of intact free-floating islets isolated from the pancreas of a 12-week-old human fetus. The culture is 2 weeks old and has been maintained in CMRL-1066 enriched with 10 percent human cord serum. Note the islet capsule and the variety of islet size (×320). (b) Intermediate-power phase micrograph of an islet from a 12-week-old human fetus; the islet was exposed for 24 hours to CMRL-1066 medium supplemented with 10 percent fetal calf serum. The capsule of the centrally located attached islet has disappeared and epithelioid granule-containing cells have fanned out to form a monolayer. There are no contaminating fibroblasts (×320).

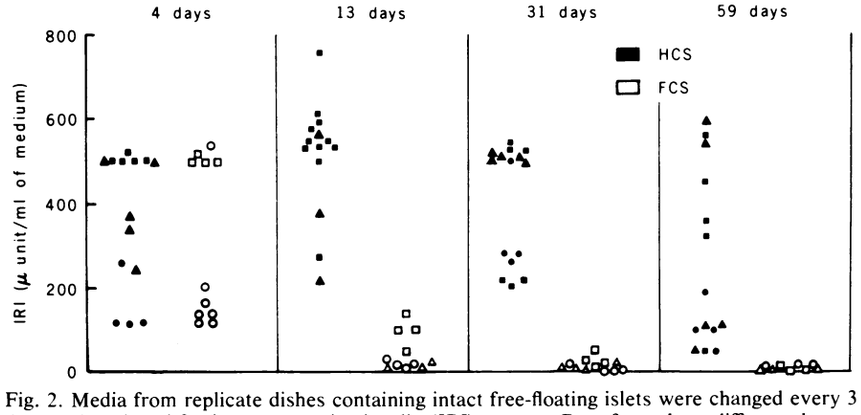

Fig. 2. Media from replicate dishes containing intact free-floating islets were changed every 3 days and analyzed for immunoreactive insulin (*IRI*) content. Data from three different donors (represented by different symbols) are summarized. Differences were significant ($P < .01$) at 13, 31, and 59 days. Glucagon content of the media was assayed at 7, 38, and 80 days. There was no difference between cultures maintained in human cord serum (HCS) and fetal cord serum (FCS) at 7 days. At 38 days, the mean glucagon content for the HCS replicates were 422, 760, and 347 pg/ml, and for the FCS replicates 33, 155, and 133 pg/ml. At 80 days all media that contained insulin still had measurable glucagon.