P. K. Clausen,[1] M.S.F.S. and W. F. Rowe,[1] Ph.D.

Differentiation of Fetal and Adult Bloodstains by Pyrolysis–Gas-Liquid Chromatography

ABSTRACT: A pyrolysis–gas-liquid chromatographic method has been developed for the differentiation of adult and fetal bloodstains. In a blind-coded study, five adult and three fetal bloodstains were correctly identified on the basis of the pyrograms of stain extracts. The differentiation between adult and fetal bloodstains is based on the peak height ratio of two long-retention-time peaks appearing in their pyrograms. The first of these peaks has been tentatively identified as indole derived from the pyrolysis of tryptophan, while the second peak is an as-yet unidentified molecular fragment produced by the pyrolysis of some component of the hemoglobin molecule other than the amino acids tryptophan, tyrosine, and phenylalanine.

KEY WORDS: pathology and biology, chromatographic analysis, blood

The ability to distinguish between variant forms of hemoglobin is of considerable importance to forensic serologists. The presence of sickle cell hemoglobin is a useful genetic marker for the individualization of bloodstains. On the other hand, in cases involving abortion or infanticide, the identification of fetal hemoglobin in bloodstains is of value.

The structural differences between adult hemoglobin (HbA), sickle cell hemoglobin (HbS), and fetal hemoglobin (HbF) are now understood [1]. Hemoglobin molecules are composed of four linear amino acid chains each bound to an iron porphyrin prosthetic group. HbA contains two α chains of 141 amino acid residues each and two β chains of 146 amino acid residues each. HbS differs from HbA only in the substitution of a valine for glutamic acid in Position 6 of the β chain. HbF differs substantially more from HbA than does HbS: HbF consists of two α chains that are identical to the α chains in HbA and two γ chains having the same number of amino acid residues as the β chains but differing from the β chains at 39 positions in their amino acid sequence. These compositional differences between the β and γ chains produce the overall compositional differences shown in Table 1.

The differences between the structures of HbA and HbF result in differences in a variety of properties: electrophoretic mobility, serological specificity, and resistance to alkali denaturation. These differences in properties form the basis for the present methods for distinguishing adult and fetal bloodstains. HbA and HbF may be separated by electrophoresis [2,3]; however, the separation presently obtainable is small compared with the separation obtained with other hemoglobin types and may be obscured by the smear-

TABLE 1—*Amino acid compositions of adult and fetal hemoglobin.*

| Amino Acid | Protein Chains | | | Adult $\alpha_2\beta_2$ | Fetal $\alpha_2\gamma_2$ |
	α	β	γ		
Alanine[a]	21	15	11 or 12	72	64 or 66
Arginine	3	3	3	12	12
Asparagine	4	6	6	20	20
Aspartic acid	8	7	7	30	30
Cysteine	1	2	1	6	4
Glutamic acid	4	8	8	24	24
Glutamine	1	3	4	8	10
Glycine[a]	7	13	13 or 12	40	40 or 38
Histidine	10	9	7	38	34
Isoleucine	...	...	4	...	8
Leucine	18	18	17	72	70
Lysine	11	11	12	44	46
Methionine	2	1	2	6	8
Phenylalanine[b]	7	8	8	30	30
Proline	7	7	4	28	22
Serine	11	5	11	32	44
Threonine	10	7	10	34	40
Tryptophan[b]	1	2	3	6	8
Tyrosine[b]	2	3	2	10	8
Valine	13	18	13	62	52

[a]Amino acid residue #136 of the γ chain may be either alanine or glycine.
[b]Aromatic amino acid.

ing of the migrating bands that result from the aging of the bloodstain. Antigen-antibody reactions may be carried out between HbF and anti-human fetal hemoglobin F antiserum using the Ouchterlony double diffusion procedure [4], electroimmunodiffusion [4], electrophoresis [5], or radial immunodiffusion [6]. HbA is more rapidly denatured by alkali than is HbF; therefore, HbF may be identified in a bloodstain extract by comparing the visible spectra of the extract both before and after addition of alkali [3].

Bayer et al [7,8] have demonstrated that HbA, HbS, and HbF may be differentiated by pyrolysis–gas-liquid chromatography (pyrolysis-GLC). Bayer and his co-workers found that the pyrolysis chromatograms (usually referred to as pyrograms) produced by HbA and HbF differed significantly: one very small short-retention-time peak was found in pyrograms of HbF and not in pyrograms of HbA; the peak height ratios of three long-retention-time peaks were found to be different in pyrograms of HbF from the values found in pyrograms of HbA. Danielson and his co-workers [9,10] have demonstrated that the major pyrolysis fragments of proteins are aromatic or heteroaromatic compounds derived from the degradation of tryptophan, tyrosine, and phenylalanine. Aliphatic amino acids were found to make no significant contribution to the long-retention-time peaks in the pyrograms of proteins. As indicated in Table 1, HbA and HbF have the same number of phenylalanine residues but differ in their relative amounts of tyrosine and tryptophan. A reasonable hypothesis is that the differences in the pyrograms of HbA and HbF reflect differences in the number of tyrosine or tryptophan residues.

The differentiation of HbA and HbF by pyrolysis-GLC suggested a new method for the differentiation of adult and fetal bloodstains. Yüregir [6] has reported that HbF comprises between 35% and 95% of the hemoglobin in fetal cord blood. Other workers [11,12] have reported ranges of HbF levels in fetal cord blood that fall within the limits reported by Yüregir. If the pyrolysis pattern of hemoglobin dominates the pyrograms of whole blood

and bloodstain extracts, then the pyrograms obtained from adult and fetal bloods or from extracts of adult and fetal bloodstains should be distinguishable. Furthermore, the differentiation should be possible by using the same peaks as those used by Bayer et al [7,8] to distinguish HbA from HbF.

The application of pyrolysis-GLC to the analysis of biological materials of interest to forensic science has been limited. DeForest [13] and Porter and Fouweather [14] have attempted to individualize human hair using pyrolysis-GLC; however, these attempts were unsuccessful because of both a lack of distinguishing features in the pyrograms produced by the hair of different individuals and significant variations in the pyrograms produced by different hairs of the same individual. Nevertheless, recent applications of pyrolysis-GLC in microbiology, entomology, and biochemical analysis suggest that this technique will find wider application in the examination of evidentiary materials of biological origin. A number of workers have explored the chemotaxonomy of microorganisms with pyrolysis-GLC. Pyrolysis-GLC has been applied to multicellular organisms (such as cockroaches) and to the characterization of mammalian cells. An analytical scheme has been proposed for the categorization of biochemical substances such as proteins, carbohydrates, lipids, and nucleic acids based on their pyrograms. Maugh [15] has recently described these developments in a comprehensive review.

The ability of pyrolysis-GLC to differentiate similar biological materials such as HbA and HbF and the availability of this technology in forensic science laboratories suggested to us the value of developing a method for the differentiation of adult and fetal bloodstains by differences in their pyrograms. We have developed a technique for the differentiation of adult and fetal whole bloods and bloodstains using pyrolysis-GLC. To test the accuracy of our method, a blind-coded study has been performed in which an analyst was required to correctly identify adult and fetal bloodstains using pyrograms of saline extracts of the stains. Finally, we have tentatively identified two of the three peaks in the pyrograms used in the differentiation.

Experimental Procedure

Human whole blood samples and saline extracts were pyrolyzed in quartz tubes with the platinum coil probe of a Chemical Data Systems CDS 190 pyroprobe. All samples were pyrolyzed at 900°C for 10 s with the pyrolyzer ramp off. The resulting pyrolysis products were separated with a Perkin-Elmer 3920B gas chromatograph equipped with flame ionization detectors. The nitrogen carrier gas and the hydrogen and compressed air for the flame ionization detectors were filtered through Alltech Associates gas purifiers to remove traces of water and compressor oil. The carrier gas was also passed through an Alltech Associates Oxy-Trap, which removes traces of residual oxygen. The gas chromatography column was a 3.2-mm (⅛-in.) outside diameter stainless steel column packed with 3.5% Carbowax 20M on Chromosorb G-AW 100-120 mesh. The flow rate of the nitrogen carrier gas was 30 mL/min; the column oven was programmed for 4 min at 60°C, followed by a linear temperature increase at the rate of 4°C/min to a final temperature of 185°C that was held for 32 min.

Fetal cord blood samples containing ethylenediaminetetraacetic acid (EDTA) as an anti-clotting agent were obtained from The George Washington University Hospital.[2] Adult blood samples were obtained by finger prick from students and staff of The Department of Forensic Sciences. In order to verify that EDTA added to the fetal cord blood did not contribute to the pyrolysis patterns observed, an adult blood sample was drawn from one of the authors (P. C.) into a Vacutainer® containing EDTA. Pyrograms were obtained

from saline extracts of a stain prepared from this preserved blood and from a stain prepared from untreated adult blood from the same individual.

Bloodstains were prepared by applying fluid human whole blood to white cotton cloth. Saline extracts were prepared by soaking 3- by 2-mm stained sections in 20 μL of physiological saline for 20 to 24 h. The blood extracts were applied to the center interiors of quartz pyrolysis tubes and dried in a 37°C incubator for at least 12 h. Whole blood samples were prepared for pyrolysis by placing 5 μL of blood in the center interiors of quartz pyrolysis tubes and drying them for several hours at 65°C in an incubator.

To assess the reliability of pyrolysis-GLC for the differentiation of fetal and adult bloods, a blind-coded study was undertaken. A total of eight bloodstains were prepared; the number of fetal and adult bloodstains comprising the eight bloodstains was determined by consulting a random number table. As Diaconis [16] has pointed out in another context (namely, establishing the protocols for experiments testing alleged extrasensory perception) the results of such blind-coded experiments may be seriously biased if the participants are cued about the structure of the experiment. In the present experiment, if the person evaluating the pyrograms and identifying the pyrolyzed samples as either adult or fetal bloodstains were aware that equal numbers of stains of each type were represented, and if all but one sample were correctly identified, then the remaining sample could be correctly identified without reference to its pyrograms. If the pyrograms in question were ambiguous (or if they would have led to an incorrect identification of the sample) if they had been evaluated in a hypothetically totally unbiased fashion, the nature of the sample would still have been correctly identified, causing a higher reliability than is proper to be ascribed to the analytical procedure. Use of a random number table resulted in five adult bloodstains and three fetal bloodstains being used in the blind-coded study.

Each stain was randomly assigned an identifying letter. Triplicate saline extracts of each bloodstain were analyzed with the pyrolysis and chromatographic conditions previously described. To further avoid possibilities of cueing, the extracts were analyzed in random order. The resulting pyrograms, identified only by the letter designations assigned to each bloodstain, were examined by a third person[3] who had had no previous contact with this project. A protocol was provided that described how to calculate the relevant peak heights and their ratios; the protocol instructed the examiner to designate each sample as either adult or fetal in origin.

Samples of phenylalanine, tyrosine, and tryptophan were also subjected to pyrolysis so that their pyrolysis products might be compared with the pyrolysis products of whole blood and bloodstain extracts. Solutions of indole and skatole in methanol (1 mg/100 mL) were subjected to gas chromatography using the same column, the same temperature program, and the same carrier gas flow rate as were used for the pyrolysis experiments.

Results and Discussion

Representative pyrograms of adult and fetal whole blood are shown in Fig. 1. These pyrograms are similar to those obtained by Bayer et al [7,8] from purified HbA and HbF and to those obtained by Reiner and Hicks [17] from red blood cells. The peaks labeled 1, 2, and 3 in Fig. 1 correspond to the long-retention-time peaks used by Bayer [8] as the basis for the differentiation of HbA and HbF; the small short-retention-time peak observed by Bayer et al [7,8] was not observed either in the pyrograms of fetal whole blood or in the pyrograms of saline extracts of dried fetal bloodstains discussed below. Table 2 shows the peak height ratios calculated for the labeled peaks. The ratio (Peak 2/Peak 1) is significantly larger for adult blood than for fetal blood. Application of student's t test indicates
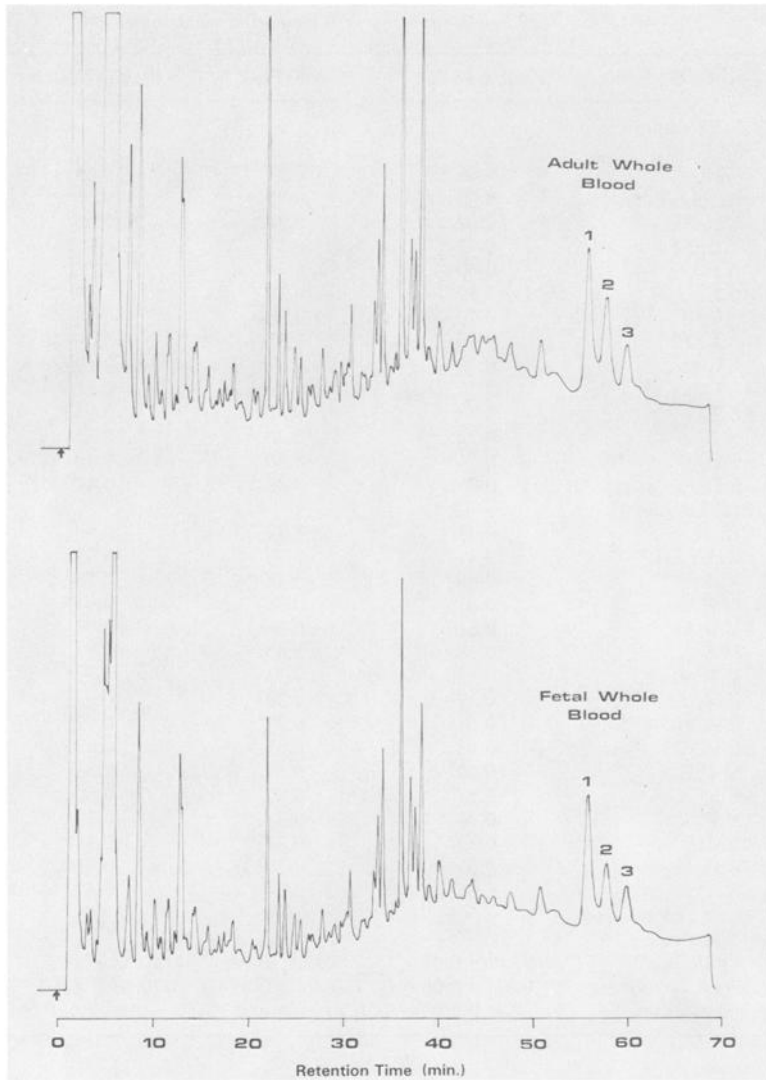
FIG. 1—*Pyrograms of adult and fetal whole blood.*

that this difference is significant at the 99.9% confidence level. Examination of Table 3 indicates that several adult and fetal samples have similar (Peak 2/Peak 1) ratios. Although at first glance this would seem to suggest that differentiation of adult and fetal bloods by pyrolysis-GLC may not be highly reliable, examination of Fig. 2 in which the (Peak 2/Peak 1) ratios are plotted in order of analysis shows that the apparent overlap of adult and fetal peak height ratios is due to a progressive increase in the (Peak 2/Peak 1) ratio for both adult and fetal blood. This increase is presumably due to a column aging effect produced by the gradual accumulation of high molecular weight pyrolysis products at the injector end of the column. In any short sequence of analysis, adult and fetal bloods could be readily differentiated.

The ratios (Peak 3/Peak 1) and (Peak 3/Peak 2) were also obtained. As indicated in Table 2, the differences in the (Peak 3/Peak 1) ratios between adult and fetal whole bloods

TABLE 2—*Peak height ratios for fetal and adult whole bloods.*

	Peak 2/Peak 1	Peak 3/Peak 1	Peak 3/Peak 2
Adult Samples			
W.R. #1	0.62	0.45	0.73
W.R. #2	0.68	0.34	0.51
R. #4	0.66	0.45	0.68
P.C. #1	0.64	0.38	0.59
P.C. #2	0.73	0.37	0.51
A.H.	0.60	0.39	0.65
N.L.	0.69	0.34	0.49
A.L. #1	0.70	0.38	0.54
A.L. #2	0.76	0.37	0.49
J.S. #1	0.76	0.37	0.49
J.S. #2	0.78	0.40	0.51
F.T.	0.68	0.37	0.54
N.R.	0.72	0.38	0.53
C.H.	0.74	0.36	0.49
P.H.	0.76	0.36	0.47
Fetal Samples			
R. #1	0.48	0.43	0.90
R. #3	0.41	0.39	0.95
V.B.	0.48	0.36	0.75
M.P.	0.57	0.35	0.61
V.B.A.	0.51	0.38	0.75
M.S.	0.58	0.43	0.74
H.R. #1	0.63	0.34	0.54
H.R. #2	0.57	0.38	0.67
L.P. #1	0.51	0.37	0.72
L.P. #2	0.58	0.37	0.64
M.P.E.	0.60	0.39	0.65
E.S.	0.53	0.32	0.60
H.L.	0.44	0.33	0.75
J.M.	0.57	0.33	0.58
B.H.	0.55	0.28	0.51
H.Y.	0.60	0.34	0.57
Means and standard deviations			
Adult	0.70 ± 0.05	0.38 ± 0.04	0.55 ± 0.08
Fetal	0.53 ± 0.06	0.36 ± 0.04	0.68 ± 0.12
Student's t test	$P < 0.001$	$0.20 < P < 0.30$	$P < 0.001$

were significant only at the 70% confidence level. The differences in the (Peak 3/Peak 2) ratio, on the other hand, were significant at the 99.9% confidence level. However, the standard deviation in the (Peak 3/Peak 2) ratio for fetal blood is approximately equal to the difference between the mean values of the (Peak 3/Peak 2) ratio for adult and fetal whole bloods. The ratios (Peak 3/Peak 1) and (Peak 3/Peak 2) are thus less reliable than the ratio (Peak 2/Peak 1) for differentiating adult and fetal whole bloods.

Figure 3 shows representative pyrograms of saline extracts of adult and fetal bloodstains. The pyrograms are essentially the same as those obtained for the whole blood samples. These results indicate that the lipid components of the blood cells (which are not extractable from the bloodstains into saline) make no contribution to the observed pyrograms of the whole blood samples. The peak height ratios for Peaks 1, 2, and 3 were again calculated and are shown in Table 3. Again, the differences in the peak height ratio (Peak 2/Peak 1) between adult and fetal blood were significant at the 99.9% level. As the data show, adult and fetal bloodstains may be readily differentiated on the basis of pyrograms obtained from saline extracts; however, contaminant peaks began to be observed in pyrograms of

TABLE 3—*Peak height ratios of stain extracts.*

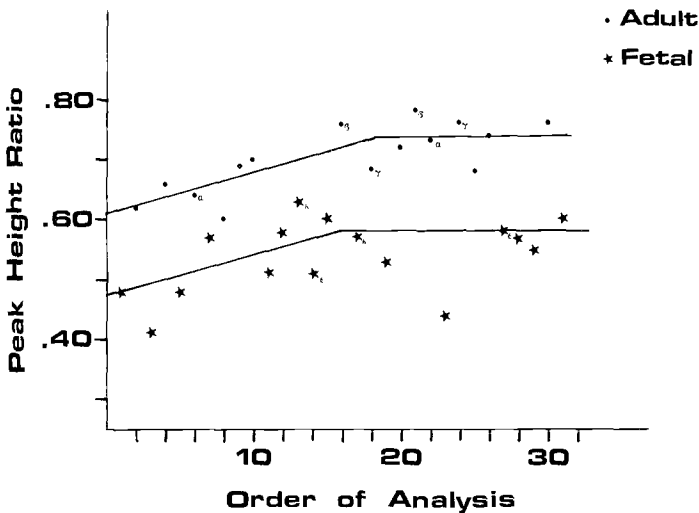| Sample	Peak 2/Peak 1	Peak 3/Peak 1	Peak 3/Peak 2
	Adult Blood		
J.B.	0.57	0.49	0.86
	0.63	0.44	0.70
	0.74	0.48	0.65
	$\overline{X} = 0.65$	$\overline{X} = 0.47$	$\overline{X} = 0.74$
C.H.	0.64	0.52	0.81
	0.68	0.50	0.74
	0.61	0.46	0.75
	$\overline{X} = 0.64$	$\overline{X} = 0.49$	$\overline{X} = 0.77$
P.C.	0.51	0.45	0.88
	0.60	0.43	0.72
	0.54	0.39	0.72
	$\overline{X} = 0.55$	$\overline{X} = 0.42$	$\overline{X} = 0.77$
A.L.	0.53	0.47	0.89
	0.50	0.46	0.92
	0.63	0.44	0.70
	$\overline{X} = 0.55$	$\overline{X} = 0.46$	$\overline{X} = 0.84$
L.W.	0.76	0.45	0.59
	0.67	0.49	0.73
	0.48	0.41	0.85
	$\overline{X} = 0.64$	$\overline{X} = 0.45$	$\overline{X} = 0.70$
W.R.	0.72	0.44	0.61
	0.60	0.47	0.78
	0.56	0.43	0.77
	$\overline{X} = 0.63$	$\overline{X} = 0.45$	$\overline{X} = 0.72$
	Fetal Blood		
C.D.	0.39	0.36	0.92
	0.45	0.35	0.78
	0.46	0.40	0.87
	$\overline{X} = 0.43$	$\overline{X} = 0.37$	$\overline{X} = 0.86$
D.C.	0.51	0.40	0.78
	0.49	0.42	0.86
	0.51	0.43	0.84
	$\overline{X} = 0.50$	$\overline{X} = 0.41$	$\overline{X} = 0.83$
G.F.	0.60	0.51	0.85
	0.50	0.41	0.82
	0.48	0.40	0.83
	$\overline{X} = 0.53$	$\overline{X} = 0.44$	$\overline{X} = 0.83$
N.K.	0.51	0.43	0.84
	0.47	0.40	0.85
	0.48	0.38	0.79
	$\overline{X} = 0.49$	$\overline{X} = 0.40$	$\overline{X} = 0.83$
D.P.	0.55	0.43	0.78
	0.40	0.36	0.90
	0.43	0.35	0.81
	$\overline{X} = 0.46$	$\overline{X} = 0.38$	$\overline{X} = 0.83$
G.P.	0.63	0.48	0.76
	0.45	0.39	0.87
	0.41	0.39	0.95
	$\overline{X} = 0.50$	$\overline{X} = 0.42$	$\overline{X} = 0.86$
Means and standard deviations			
Adult	0.61 ± 0.04	0.46 ± 0.02	0.76 ± 0.05
Fetal	0.49 ± 0.03	0.40 ± 0.03	0.84 ± 0.01
Student's *t* test	$P < 0.001$	$0.001 < P < 0.01$	$P < 0.001$

FIG. 2—*Whole blood Peak 2/Peak 1 peak height ratios plotted in order of analysis. Identical letters indicate replicate samples.*

extracts of both adult and fetal bloodstains. The peaks took the form of one or more large peaks with retention times similar to Peaks 1, 2, and 3. Since these peaks appeared in the first (and occasionally also the second) pyrogram of a series run after a protracted shutdown of the chromatograph (overnight or longer), it was concluded that they resulted from the gradual accumulation of protein degradation products on the injector end of the column. These peaks appeared even in pyrograms obtained without an analytical sample, when just a clean pyrolysis tube was used. Reconditioning the column after each series of pyrograms reduced the size of the contaminant peaks but did not completely eliminate them. All pyrograms in which contaminant peaks were observed were discarded and were not used in any subsequent analysis.

The difference in the (Peak 3/Peak 2) ratio for adult and fetal bloodstain extracts was significant at the 99.9% confidence level, while the differences in the (Peak 3/Peak 1) ratio was significant at the 99% confidence level. The difference in the means of the (Peak 2/Peak 1) ratio is three times the largest standard deviation in the means, while the corresponding differences for the ratios (Peak 3/Peak 1) and (Peak 3/Peak 2) are only twice the largest standard deviation. For this reason, the (Peak 2/Peak 1) ratio was used in the double-blind study as the most reliable basis for the differentiation of adult and fetal bloodstain extracts.

Table 4 shows the results of the pyrolysis of extracts of adult bloodstains that were made with adult blood that had been treated with EDTA as a preservative and bloodstains that were made with untreated adult blood. These results indicate that the differences observed between the pyrograms of adult and fetal bloodstains are not due to the presence of preservatives in the fetal cord blood samples.

The results of the blind-coded study are shown in Table 5. The adult and fetal bloodstains were identified with 100% accuracy. The pyrograms of two adult bloodstain extracts (one for Stain C and one for Stain H) displayed peak height ratios in the fetal bloodstain extract range. This illustrates the problem of reproducibility often encountered in pyrolysis-GLC work: closer attention to positioning the pyrolysis tubes and use of disposable precolumns may eliminate this source of error.
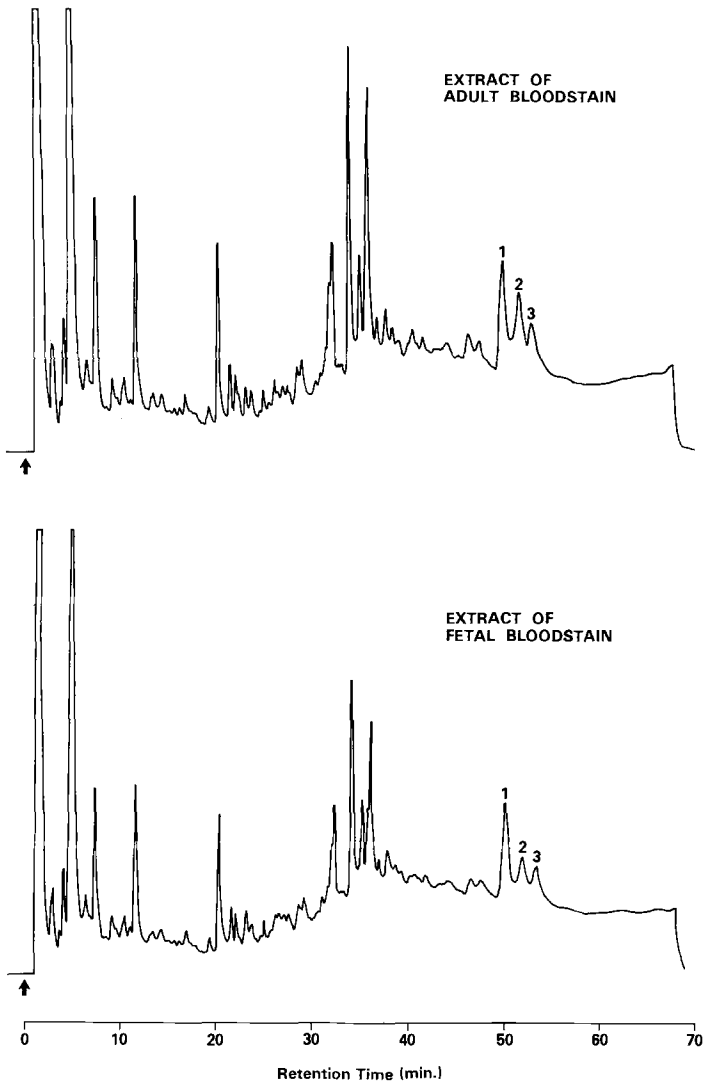
FIG. 3—*Pyrograms of adult and fetal bloodstain extracts.*

TABLE 4—*Peak height ratios of stain extracts from preserved and fresh adult blood.*

	Peak 2/Peak 1	Peak 3/Peak 1	Peak 3/Peak 2
A1	0.65	0.41	0.63
A2	0.82	0.44	0.54
A3	0.74	0.47	0.64
	$\overline{X} = 0.74$	$\overline{X} = 0.44$	$\overline{X} = 0.60$
B1	0.80	0.45	0.56
B2	0.66	0.40	0.61
B3	0.84	0.44	0.52
B4	0.65	0.44	0.68
	$\overline{X} = 0.74$	$\overline{X} = 0.43$	$\overline{X} = 0.59$

TABLE 5—*Analyst's determination of bloodstain type in a blind-coded study.*

Sample	Type of Stain	Peak 2/Peak 1	Identity Assigned by Analyst
A	fetal (J.F.)	0.42	fetal
		0.54	
		0.45	
		$\overline{X} = 0.48$	
B	adult (P.C.)	0.65	adult
		0.68	
		0.77	
		$\overline{X} = 0.70$	
C	adult (S.B.)	0.71	adult
		0.51	
		0.81	
		$\overline{X} = 0.68$	
D	adult (F.T.)	0.78	adult
		0.61	
		0.58	
		$\overline{X} = 0.66$	
E	adult (C.H.)	0.88	adult
		0.59	
		0.58	
		$\overline{X} = 0.68$	
F	fetal (P.M.)	0.45	fetal
		0.39	
		0.40	
		$\overline{X} = 0.41$	
G	fetal (B.L.)	0.56	fetal
		0.49	
		0.50	
		$\overline{X} = 0.52$	
H	adult (A.L.)	0.51	adult
		0.58	
		0.68	
		$\overline{X} = 0.59$	

Understanding the origin of the differentiation of adult and fetal bloods using pyrolysis-GLC requires the identification of the molecular fragments representing Peaks 1, 2, and 3. The pyrolysis of the amino acid tryptophan yields two long-retention-time peaks corresponding to Peaks 1 and 3 (Fig. 4). The retention times of indole and skatole correspond to the retention times of Peaks 1 and 3, respectively (Fig. 5). It seems reasonable, therefore, to identify tentatively Peak 1 as indole and Peak 3 as skatole, with both of these compounds being produced by the pyrolysis of tryptophan. Phenylalanine also contributes to Peak 1, as indicated by pyrograms of this compound (Fig. 4). However, under the pyrolysis conditions of these experiments, the contribution of phenylalanine to Peak 1 is insignificant. Tyrosine contributes no peaks to this region of the pyrograms (Fig. 4). The origin of Peak 2 remains uncertain. If, however, Peak 2 derives from a component of the hemoglobin molecule that is present in the same quantity in both HbA and HbF, the observed values of the (Peak 2/Peak 1) ratio are readily explained. As Table 1 indicates, adult hemoglobin contains less tryptophan than does fetal hemoglobin; therefore, the (Peak 2/Peak 1) ratio should be larger for adult blood than the (Peak 2/Peak 1) ratio for fetal blood. If f represents the fraction of fetal hemoglobin in the fetal blood samples, then it is easy to show that
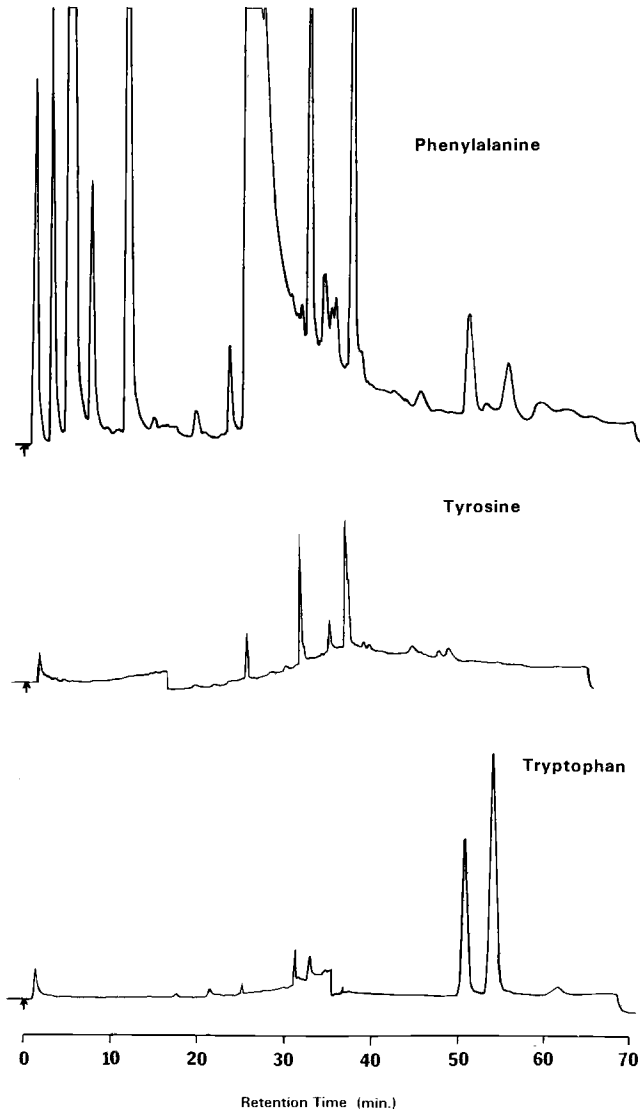
FIG. 4—*Pyrograms of phenylalanine, tyrosine, and tryptophan.*

$$\frac{(\text{Peak 2/Peak 1) fetal}}{(\text{Peak 2/Peak 1) adult}} = \frac{6}{6 + 2f}$$

neglecting the contribution of phenylalanine to Peak 1.

Reported values of f [6,11,12] range from 0.35 to 0.95, resulting in values for the above ratio of 0.90 and 0.76, respectively. The observed value for the pyrograms of the blood-stain extracts is 0.80 ± 0.10, while the observed value for the pyrograms of the whole blood samples is 0.76 ± 0.14. The expected values fall within one standard deviation of the observed values. On the other hand, if the observed value of the ratio of peak height ratios is used to calculate an average f value, one obtains an f of 0.75 for the fetal blood-

FIG. 5—*Chromatograms of 1 mg/100 mL solutions of indole, skatole, and indole plus skatole.*

stain extracts and an f of 0.95 for the whole blood samples. An average f value of 0.80 has been reported [11].

Additional research is required before pyrolysis-GLC can be considered for routine differentiation of adult and fetal blood in the forensic science laboratory. Among the questions that will be addressed in subsequent work will be the sensitivity limit of this procedure and the effect of aging on the pyrograms of bloodstain extracts. The electrophoretic and immunological methods for the differentiation of adult and fetal bloodstains can be applied to very small stains. The minimum size of bloodstain to which pyrolysis-GLC can be readily applied has not yet been determined. The authors think that the sample size may be reduced by a factor of 2 and perhaps by a factor of 4 without adversely affecting results.

The minimum size of bloodstain that can be analyzed by pyrolysis-GLC will be affected by the degradation of hemoglobin in the bloodstain. Such degradation occurs as bloodstains age. Subsequent research will examine the effect of aging on the peak height ratios for Peaks 1, 2, and 3. The effect of bloodstain aging on the pyrograms of saline extracts is a worthwhile research area in its own right. Aging may produce changes in the pyrograms in some regular and reproducible fashion so that the age of a bloodstain may be inferred from pyrograms of the bloodstain's extract.

It would also be valuable to establish conclusively the identities of Peaks 1, 2, and 3. Subsequent research will be directed at discovering the source of Peak 2 in the hemoglobin molecule and determining its identity.

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Address request for reprints or additional information to
Walter F. Rowe, Ph.D.
Department of Forensic Sciences
The George Washington University
Washington, D.C. 20052