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Secreted Blood Group Substances: Distributions in Semen and Stabilities in Dried Semen Stains

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ABSTRACT: A sensitive microplate hemagglutination-inhibition technique has been used to ascertain the distributions of secreted blood group substances (BGS) in a population of 176 semen specimens and to characterize the stability of these substances in dried semen stains. The BGS concentrations in semen were found to vary throughout a wide range of titer. Despite this latitude of variation, the titers for the component BGS within the blood groups could be described by a log-normal distribution function. Studies of a number of sequential semen specimens obtained from the same donors revealed that the intraindividual variation in BGS titers was much more limited than the interindividual BGS titers. Attempts to correlate variations in titers between A and H in Group A semen or B and H in Group B semen indicated that the levels of these component substances vary independently.

Studies of the stability of BGS in Groups A and O semen suggested that these substances were stable when the semen stains were stored at −20°C, 4°C, or at ambient laboratory temperature in a dry state. In contrast, stains stored at 37°C under humid conditions suffered a dramatic loss in BGS titer, with the half-life of the BGS being on the order of 30 days.

KEYWORDS: pathology and biology, genetic typing, semen, secreted blood group substances, microplate hemagglutination-inhibition test

Dried body fluid stains characterize evidential materials from sexual assault cases. A cardinal step in the forensic science analysis of stain evidence is an attempt to ascertain the blood group of the fluid depositor. The elaboration of the soluble blood group substances (BGS), A, B, and H, into the body fluids of secretor individuals fortuitously provides a means by which these fluids may be partially individualized when they are present as stains.

Frequently the interpretation of grouping test results of stained specimens can be enhanced by an appreciation of the levels of the A/B/H BGS in the body fluids of the normal population. Such information can provide a valuable insight as to the relationships among the individual BGS which are present in each body fluid. There have been several studies in which the A/B/H BGS titers were measured in semen or saliva or both [*1-3*].

Although the ranges of BGS titer values in these fluids have varied from study to study, much of the variation can be attributed to different degrees of detection sensitivity. In general, the average titers of A BGS or B BGS exceed the titers of H BGS in the semen and saliva from

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secretor individuals of blood groups A, B, or AB. McNeil et al [*4*] and others [*5*] have presented evidence that a small population of individuals, termed aberrant secretors, will exhibit higher levels of H BGS than A or B BGS in their saliva. In contrast, reports from the Home Office Central Research Establishment[2] [*6*] indicate that H BGS titers almost always exceed those of A or B BGS for both semen and saliva. Clearly, there is a conflict among these studies as to the ratios of A:H and B:H which one would expect to find in semen.

Our recent development of a more sensitive inhibition method for the measurement of BGS in body fluids was accompanied by a comprehensive reexamination of the levels of the A/B/H BGS in semen as well as their stabilities in dried semen stains. The results of this study support the position that A or B BGS levels in semen most frequently exceed those of H BGS.

Materials

Semen Specimens

Semen specimens were obtained from normal donors through cooperating area fertility clinics. All semen contributors were healthy individuals with no known history of genitourinary pathology. Donor ages ranged from 21 through 40 years. Semen was collected in sterile containers and stored at −70°C until analyzed. The Lewis phenotype of each specimen had been determined as part of a companion study [*7*].

Antisera, Lectin, and Indicator Erythrocytes

Anti-A and anti-B blood grouping antisera, as well as indicator red blood cells (Affirmagen and Selectogen), were obtained from Ortho Diagnostics, Raritan, NJ. H-lectin (Ulex europaeus) was purchased from Serological Research Institute, Emeryville, CA.

Miscellaneous Materials

N-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid (HEPES), bovine serum albumin, and Tween-20 were obtained from Sigma Chemical Company, St. Louis, MO. All grouping tests were performed in 96-well, V-bottom, polystyrene microplates which were manufactured by Dynatech Industries, Alexandria, VA.

Methods

Hemagglutination-Inhibition Test

The hemagglutination-inhibition assay was adapted from published procedures [*8,9*]. Anti-A antiserum (anti-A), anti-B antiserum (anti-B), and H lectin solutions of desired strength were prepared by diluting the commercial agglutinins with a solution composed of HEPES-buffered saline (0.01*M* HEPES: 0.144*M* sodium chloride, pH 7.2; [HBS]) which contained 1% (w/v) bovine serum albumin and 1% (v/v) Tween-20 (THBS). Indicator erythrocytes (RBC) were washed twice with HBS and suspended in HBS at a final concentration of 0.5% (v/v).

Optimization of Antisera and Lectin Strengths

Anti-A, anti-B, and H-lectin were titrated in narrow increments of dilution against the appropriate RBC type to establish the minimum concentrations of these reagents necessary to effect complete agglutination of a fixed concentration of RBC. The routine agglutinin dilution

[2]M. J. Davie, M. J. Dorrill, and P. H. Whitehead, personal communication, 1979.

series were: anti-A and anti-B: 1:700, 800, 900, 1000, 2000, 3000, 4000, and 5000; H lectin: 1:200, 300, 400, 500, 600, 700, 800, 900 and 1000. Of the diluted typing sera or lectin 20 μL were combined with 10 μL of HBS in individual wells of a microplate. After mixing was accomplished with a fine stream of air, 20 μL of indicator RBC were added. Again, the contents of each well were mixed and permitted to stand at room temperature for 30 min. The microplate was centrifuged in a Dupont-Sorvall GLC-4 centrifuge at 1300 × *g* for 5 min. Presence or absence of RBC agglutination was assessed macroscopically following placement of the microplate at a 60° angle for 30 min. During the tilt phase of the assay, the RBC which were not agglutinated slowly flowed in a smooth stream toward the edge of the well. In contrast, agglutinated RBC remained as a distinct pellet at the bottom of the well. Strongly agglutinated RBC pellets occasionally detached from the well bottom and slid intact to the well edge. Partially agglutinated RBC exhibited some streaming during the tilt period, however, the streams failed to reach the well edge during the 30-min observation time. The agglutination end-points of antisera and lectin were defined as the greatest dilution of these reagents which caused the complete agglutination of the RBC.

Titrations of Semen and Semen Stain Extracts

Liquid semen specimens were centrifuged to pellet spermatozoa and debris, and the supernatant seminal fluids diluted with HBS in serial doubling dilution steps which spanned a range from 1:500 through 1:512 000. Extracts of semen stains were diluted with HBS from 1:100 through, 1:25 600. Testing of these samples was identical to the protocol described for optimizing the agglutinin strength except 10-μL aliquots of the semen, or semen stain extract, replaced the 10-μL HBS, and the antisera and lectin were used at strengths equivalent to two dilution steps back from their titration end points. Antisera and specimen were incubated at room temperature for 30 min before the addition of RBC.

Preparation and Extraction of Semen Stains

Semen stains were prepared by pipetting 5-μL aliquots of undiluted semen onto boiled cotton sheeting. All stains were permitted to dry overnight at room temperature and ambient humidity before their disposition to one of several conditions of storage. For each semen specimen, four panels of replicate stains were prepared. Individual panels were stored at −20°C, 4°C, ambient laboratory temperature, and 37°C. The panels at 37°C were maintained in a sealed moisture chamber; whereas all other panels were kept in airtight containers at their respective temperatures. At intervals, individual stains from each storage condition were cut out in their entirety and extracted with 100 μL of HBS for 1 h at room temperature. The levels of BGS in each stain extract were determined by titration. Each stain extract was diluted with HBS in serial doubling steps and duplicate 10-μL aliquots were used to test separately for A, B, and H BGS. By maintaining accurate volumetric control over all aspects of this study, the titers of BGS in the stain extracts could be related directly to the BGS titers in the liquid semen specimens used for stain preparation.

Expression of Titration Data

Within this paper, BGS titer has been defined as the reciprocal of the greatest dilution of a seminal fluid specimen which demonstrated the ability to inhibit RBC agglutination. All expressions of antisera dilutions and BGS titers are in terms of their final values in the microplate wells at the completion of the assay. The significance of differences in means was assessed by the student's *t*-test.

Results

Titrations of A/B/H BGS in Semen

Table 1 shows the titers of BGS that were ascertained for 176 liquid semen samples. Each specimen had been tested prior to this study and found to have originated from an individual of the Lewis (a-b+) phenotype. The blood group of each donor was inferred from the BGS grouping patterns. Several aspects of these data were salient. First, it was apparent that performance of the grouping tests in microplates had conferred considerable sensitivity upon the analysis, since the BGS titers in several specimens exceeded 500 000. Second, the titers of BGS in semen were observed to vary over an extremely wide range. A and H BGS in Group A semen exhibited the most variation in titer, 128-fold. The least variation seen was the four-fold range for H BGS in Group AB semen. The geometric mean was calculated for the individual BGS within each of the four blood groups. The mean titer for H BGS was similar among the four groups, with all means lying within a range of one doubling dilution. The mean titer for A BGS in Group A semen was about the same as seen for the mean of A BGS in AB semen. The mean titer for B BGS in Group AB semen was more than twice that for B BGS in B semen, although the few AB specimens must temper any conclusion drawn from this group.

Additional information was distilled from the BGS titer data in this study. For the 53 Group A and 28 Group B semen samples, a least-squares linear regression analysis was performed to test for statistical evidence of a correlation between the variations in BGS levels. As shown in Fig. 1, a comparison of A BGS with H BGS in Group A semen yielded a correlation coefficient of 0.27, indicating a lack of significant correlation. The same statistical treatment of the BGS titer values for Group B semen revealed a similar lack of correlation ($r = 0.13$).

It was of interest to calculate the ratios of A/H BGS for A semen and B/H for B semen. Rather than use the mean BGS titers for these calculations, which would have been inappropriate since the BGS levels were not correlated, this ratio was calculated for each separate semen specimen, and the individual ratios averaged. Using this approach, it was seen that 75% of the Group A semen samples had an A/H ratio equal to or greater than one (Table 2). The range of A/H ratios within this group was from 0.0625 to 32, and the mean was 4.6. Only one of the Group B semen specimens exhibited a lower B BGS titer than H BGS titer. The range of B/H ratios was from 0.5 to 128 and the mean was 16.

Empirically, it was determined that the BGS titer data were distributed in a log-normal fashion. The $\log_2$ values for each of the BGS in each of the groups (except AB) were taken, and these values ranked and plotted as a function of their cumulative frequency on a probability scale. As seen in Figs. 2 to 4, the $\log_2$ of the titers of the BGS present in each group described a straight line when expressed in this fashion, suggesting that the titers were log-normally distributed within these populations.

TABLE 1—*Titers of soluble blood group substances in seminal fluid.*

Blood Group	Number of Samples	Substance Present	$10^{-3} \times$ Titer	
			Mean	Range
A	53	A	20	1-128
		H	12	1-128
B	28	B	95	8-512
		H	12	4-64
		A	24	1-128
AB	7	B	195	32-512
		H	13	8-32
O	88	H	20	4-256

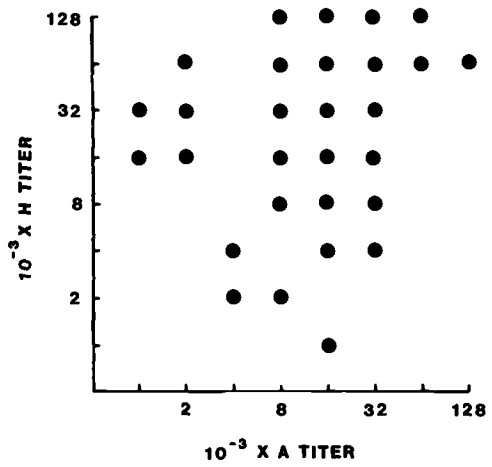

FIG. 1—*Titers of A blood group substance in Group A semen specimens plotted against their companion H blood group substance titers.*

TABLE 2—*Ratios of blood group substances in the seminal fluid of secretor individuals.*

Blood Group	A/H		B/H		Percent Specimens of Ratio ≥ 1
	Range	Mean	Range	Mean	
A	0.063-32	4.6	...	...	75
B	...	...	0.5-128	16	96

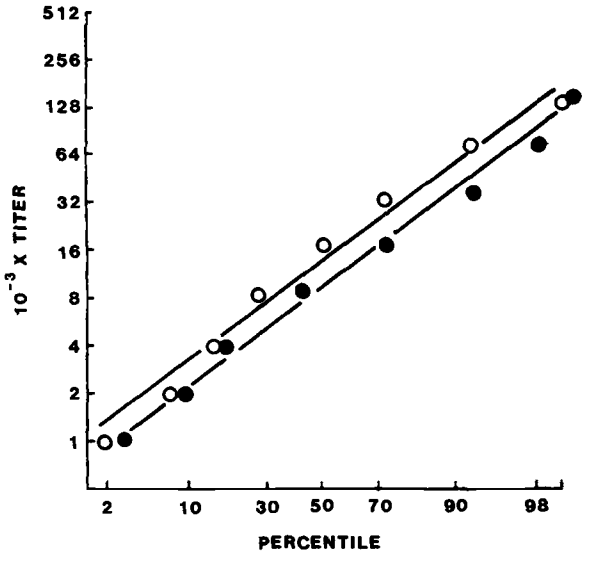

FIG. 2—*Cumulative frequency distributions for A and H blood group substances in Group A semen specimens. Open circles, A blood group substance; closed circles, H blood group substance.*

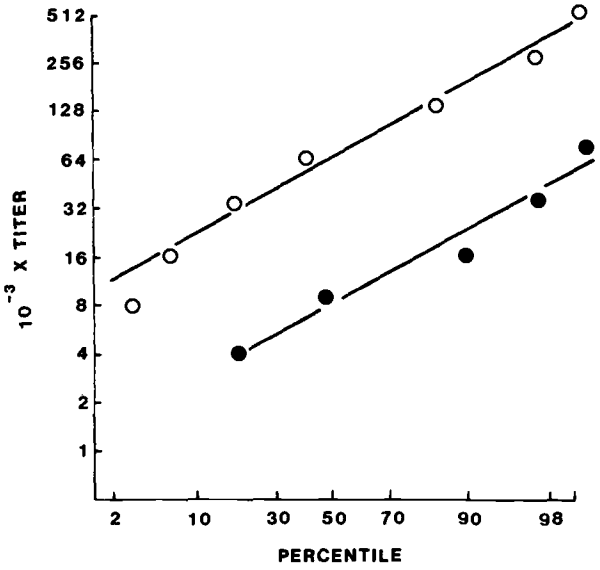

FIG. 3—*Cumulative frequency distributions for B and H blood group substances in Group B semen. Open circles, B blood group substance; closed circles, H blood group substance.*

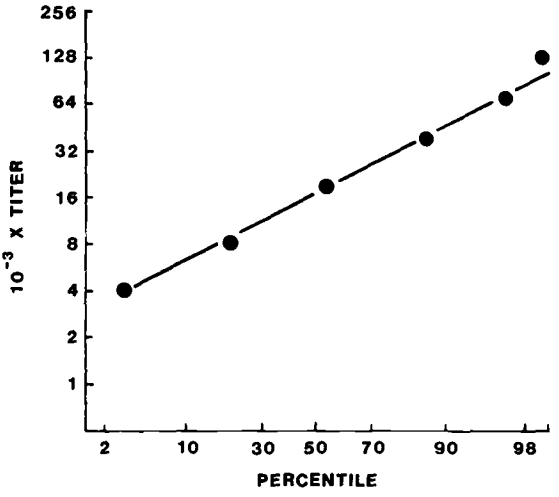

FIG. 4—*Cumulative frequency distribution for H blood group substance in Group O semen.*

Analysis of Semen from Nonsecretors

Semen specimens from seven individuals of the Lewis phenotype (a + b-) were examined for evidence of soluble BGS. No BGS were detectable under the conditions of this assay, even when the samples were analyzed undiluted.

Intraindividual Variation in Seminal Fluid BGS Titers

Table 3 illustrates the BGS titers found in separate semen samples donated by the same individuals. The interval of time between donations was not controlled, but was a minimum

TABLE 3—*Intraindividual variation in seminal fluid blood group substance titers.*

Donor	Specimen	10^{-3} × Titer		
		A	B	H
N-39	A	2	...	8
	B	8	...	8
N-38	C	1	...	16
	D	4	...	32
	E	4	...	32
N-36	A	...	64	16
	C	...	128	16
	D	...	256	8
N-27	A	...	...	16
	B	...	...	16
	C	...	...	32
N-35	A	...	...	32
	B	...	...	16
	C	...	...	16

of one week. It was apparent from the results that the intraindividual variations in BGS titers were considerably less than the interindividual variation. The most variation seen was fourfold.

Stability of BGS in Dried Semen Stains

Semen specimens from 17 donors were chosen for a study of the stability of BGS in dried semen stains stored under several different environmental conditions. Nine of the samples were Group A and eight were Group 0. At irregular intervals over a ten-month period, one stain from each specimen at each storage temperature was extracted and the BGS titers determined. Figures 5 and 6 show the survival patterns of the BGS in these stains. Rather than separately illustrate the BGS stabilities for each semen specimen, geometric means are shown which were calculated from the titration results for all of the stains of the same blood group maintained under the same storage condition. Shown also for each mean value is its standard deviation.

Throughout the study period, the BGS in dried semen stains stored at −20°C, 4°C, and at ambient laboratory temperature were remarkably stable. At each assay time point, there were no significant differences ($p > 0.1$) among the mean titers of each of the BGS in stains maintained at these three temperatures. For A BGS in Group A semen stains, the slight decrease in mean titer from Day 244 on was significantly different ($p < 0.05$) from the mean titer at Day 0. The mean H BGS titers in A semen did not decrease significantly in these stains. For the H BGS in Group O semen stains, a similar picture emerged, for the mean titers of H BGS did not decrease significantly during the observation period. Overall, the storage of semen stains at −20°C, 4°C, and at room temperature resulted in, at most, a twofold decrease in mean titer of the BGS.

In contrast, the BGS in stains stored at 37°C under humid conditions suffered a substantial decrease in mean titer over the ten-month study period. The patterns of instability, or degradation, were almost identical for A and H BGS in Group A semen and H in 0 semen. For Group 0 semen, the decrease in H titer was significant ($p < 0.05$) by Day 42; whereas, for A and H in Group A semen, a significant decrease ($p < 0.05$) was apparent only after 92 days of storage. Inspection of the curves in Figs. 5 and 6 suggests that the half-life of A and H BGS in either A or 0 semen stains stored at 37°C was about 30 days.

Although only semen specimens of blood Groups A and 0 were used in this portion of the

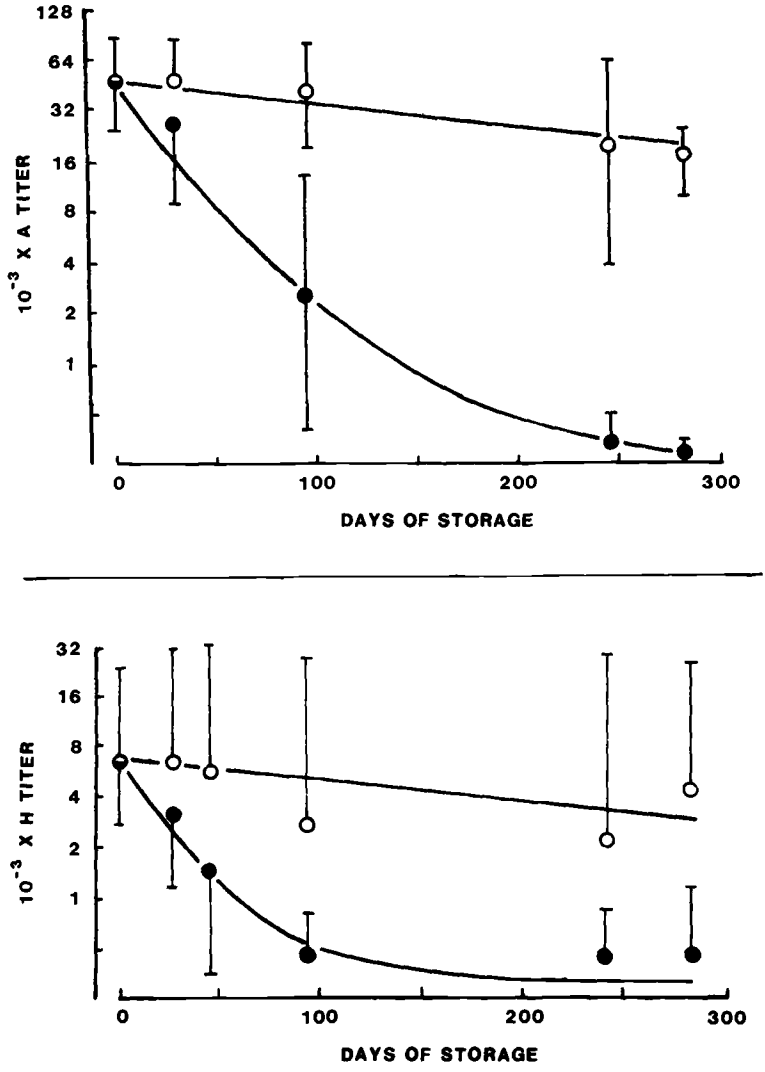

FIG. 5—*Stabilities of A and H blood group substances (BGS) in dried Group A semen stains as a function of storage time and temperature. Upper panel: A BGS; lower panel: H BGS. Open circles, stains stored at −20°C, 4°C, and ambient laboratory temperature; closed circles, stains stored at 37°C. Data points are the geometric means ± one standard deviation.*

study, all stain extracts were tested for A, B, and H BGS. None of the semen stains stored at any of the temperatures acquired an unexpected BGS during the storage period. That is, none of the A semen stain specimens ever demonstrated B BGS; nor did the Group 0 specimens demonstrate any BGS but H.

Discussion

This study was carried out to attain two goals. The immediate objective was to evaluate the sensitivity and reliability of the microplate assay for secreted BGS in human body fluids. In another sense, it was desired that this study would: (1) assist in resolving literature conflicts

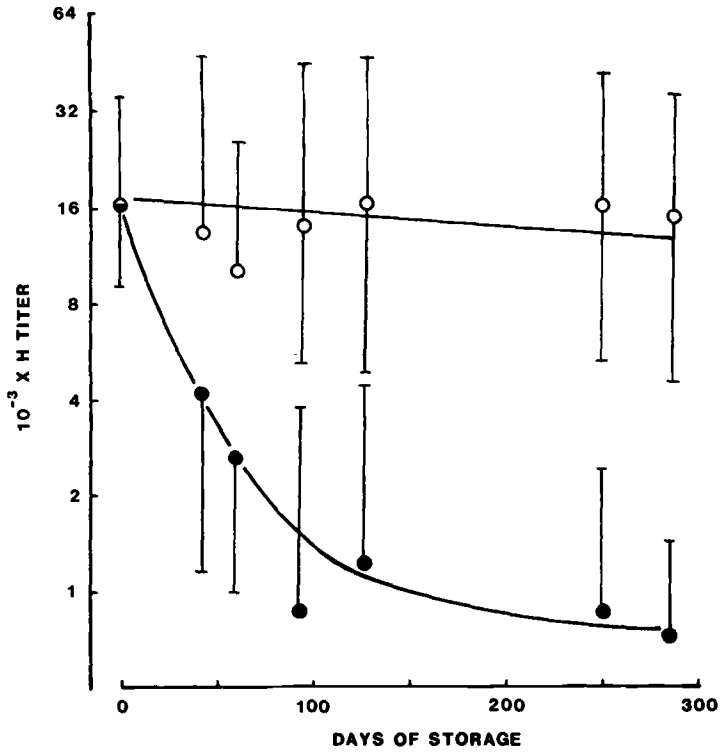

FIG. 6—*Stability of H blood group substance in dried Group O semen stains as a function of storage time and temperature. Open circles, stains stored at −20°C, 4°C, and ambient laboratory temperature; closed circles, stains stored at 37°C. Data points are the geometric means ±one standard deviation.*

regarding seminal fluid BGS ratios and (2) provide a solid data foundation for use in the interpretation of body fluid stain evidence. The results would suggest that success has been achieved in each area. The routine analysis and evaluation of semen stain evidence in our laboratory has been facilitated as a result of these studies.

BGS Titers in the Population

Several principles have emerged from this study which may be used to guide the interpretation of semen stain evidence. It was recognized that the interindividual seminal fluid BGS titers vary over a considerable range. But despite the breadth of this variation, the titers were described by a normal distribution function. Thus, basic statistical analyses may be performed on these data to enable quantitative inferences to be drawn concerning the interrelationships among seminal fluid components of forensic science interest. The observation that the BGS titer measurements fit a log-normal distribution curve was not unprecedented. Numerous biological substances, for example, serum enzymes, have been found to be log-normally distributed in the general population [*10*]. Moreover, it has been shown that the variations in both seminal fluid and vaginal fluid acid phosphatase activities follow log-normal distribution patterns [*11,12*]. While it is not clear why some population parameters best fit a normal distribution only after transformation of the measurement scale, statistical analyses upon transformed data are nonetheless appropriate [*13*].

A/B/H Ratios in Semen

An appreciation has been gained for the interrelationships among the component BGS in semen specimens. The data from this study have indicated that 3/4 of Group A secretor individuals exhibit an A BGS titer in excess of the H BGS titer. For Group B individuals, the B/H ratio was equal to or greater than one for 96% of the specimens. These findings were in harmony with the observations of a similar study by Sensabaugh et al [*2*]; yet conflict with reports by others[2] [*6*]. In the latter studies, it was reported that for seminal fluid, the A/H or B/H ratio was almost always less than one. However, the quantifications of seminal fluid BGS were standardized against pooled saliva. Since H BGS titers are low in saliva relative to semen, whereas the A or B BGS titers are about the same in these two fluids [*1,12,14*], a calibration of the grouping assay for semen against saliva would act to increase the apparent titers of H BGS in semen. Thus, it would appear to be inappropriate to use BGS titer distribution data developed using one type of body fluid for the calibration of grouping tests carried out on a different fluid.

The observation that the titers of A and H or B and H were not correlated within specimens might seem surprising. The H blood group structure is an obligate precursor to the formation of either the A or B specificities [*15*], and conversion of H to either A, B, Le a, or Le b specificities results in a masking of the H antigenic specificity [*16*]. Thus, it might be expected that as the A or B BGS titers rise, there would be a commensurate decrease in the H BGS titers. However, it has been recognized that secreted BGS molecules can possess more than one serologic specificity [*15,17*]. Thus, A, B, H, Le a, and Le b specificities may all reside on a single BGS macromolecule, depending upon an individual's genotype. BGS macromolecules of different specificity arrays may arise because of competition for substrate sites among the glycosyl transferases which catalyze the addition of chain terminating immunodominant sugars onto the glycoprotein core [*18*].

BGS in Semen of Lewis (a + b-) Individuals

There have been anecdotal reports [*19*] which suggest that secreted BGS may be found in the body fluids of individuals of the Le (a + b-) phenotype. In the present study, in which the semen from seven individuals of the nonsecretor phenotype was examined by a hemagglutination-inhibition method, no evidence of soluble BGS was observed, even when the specimens were tested without dilution. It could be argued, however, that too few specimens were tested to have enabled an encounter with a sample which showed BGS but which originated from an apparent nonsecretor.

Stability of BGS in Dried Semen Stains

The degradation patterns of BGS seen in dried stains were in concert with the general principles [*12*] which characterize the ex vivo stability of biological molecules as a function of temperature and time. Intuitively, one would anticipate that semen stains maintained under conditions which were suitable for the activity of endogenous proteolytic enzymes, or conducive to the growth of bacteria, might suffer more extensive degradation than stains stored dry at lower temperatures. In the present study, BGS in semen stains kept at 37°C, in a moist environment, exhibited a half-life of approximately one month. In contrast, the BGS in stains which remained at ambient laboratory temperature, 4°C, or −20°C, demonstrated only a twofold drop in mean titer over the ten-month period. Interestingly, both A and H BGS in Group A semen exhibited the same half-life, suggesting that there was no preferential degradation of one BGS specificity over the other. Although A and H titers in A semen decay at similar rates, one might predict that H detectability would be lost before that of A because the titer of H in seminal fluid generally is less than that of A.

This stability study does not purport to be a comprehensive representation of the survival characteristics of seminal fluid BGS in evidence specimens. Biological evidence can experience a variety of undocumentable environmental insults between the times of deposition and

collection, which no laboratory designed stability experiment can simulate. However, controlled experiments can be carried out to assess BGS stability at defined extremes of environment. Defining the survival patterns of seminal fluid BGS at extremes enables a cogent prediction of their behavior within those extremes.

Acknowledgment

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References

[1] Sato, M. and Ottensooser, F., "Blood Group Substances in Body Fluids. Comparison of the Concentrations in Semen and Saliva," *Journal of Forensic Medicine*, Vol. 14, No. 1, Jan.-March 1967, pp. 30-38.

[2] Sensabaugh, G. F., Bashinski, J., and Blake, E. T., "Titers of ABH Blood Group Substances in Human Semen," presented at the 33rd Annual Meeting of the American Academy of Forensic Sciences, Los Angeles, CA, Feb. 1981.

[3] Barrantes, R. and Salzano, F. M., "Genetic and Non-genetic Influences on the ABH and Le Antigen Levels of Saliva and Milk," *Human Heredity*, Vol. 29, 1979, pp. 230-235.

[4] McNeil, G., Trentalman, E. F., Kreutzer, V. O., and Fullmer, C. D., "Aberrant Secretion of Saliva A, B and H Group Substances in Human Beings," *American Journal of Clinical Pathology*, Vol. 28, 1957, pp. 145-151.

[5] Fiori, A., DeMercurio, D., Panari, G., and Burdi, P. R., "The ABO(H) Paradoxical and Aberrant Secretion in Human Saliva," *Forensic Science International*, Vol. 17, 1981, pp. 13-17.

[6] Dorrill, M. J., "The Identification and Distribution of ABH and Lewis Substances in Seminal and Vaginal Secretions," in *Proceedings of Forensic Science Symposium on the Analysis of Sexual Assault Evidence*, FBI Laboratory, in press.

[7] Baer, B. R. and Kearney, J. J., "A Micro-Capillary Absorption-Inhibition Methods for the Determination of Lewis Phenotypes from Semen and Saliva Stains," presented at the 35th Annual Meeting of the American Academy of Forensic Sciences, Cincinnati, OH, Feb. 1983.

[8] Crawford, M. N., Gottman, F. E., and Gottman, C. A., "Microplate System for Routine Use in Blood Bank Laboratories," *Transfusion*, Vol. 10, No. 5, Sept.-Oct. 1970, pp. 258-263.

[9] Lapinski, F. J., Crowley, K. M., Merritt, C. A., and Henry, J. B., "Use of Microplate Methods in Paternity Testing," *American Journal of Clinical Pathology*, Vol. 70, No. 5, 1978, pp. 766-769.

[10] Caputo, M. J., "The Use of Probability Plots and Log-Transformations in Normal Range Determinations," *American Journal of Medical Technology*, Vol. 38, No. 1, 1972, pp. 1-6.

[11] Sensabaugh, G. F., "The Quantitative Acid Phosphatase Test. A Statistical Analysis of Endogenous and Postcoital Acid Phosphatase Levels in the Vagina," *Journal of Forensic Sciences*, Vol. 24, No. 2, April 1979, pp. 346-365.

[12] Sensabaugh, G. F., "The Acid Phosphatase Test," in *Proceedings of Forensic Science Symposium on the Analysis of Sexual Assault Evidence*, FBI Laboratory, in press.

[13] Snedecor, G. W. and Cochran, W. G., *Statistical Methods*, Iowa State Univ. Press, Ames, 1967.

[14] Baechtel, F. S. and Shewmake, T. S. "Levels of Soluble A/B/H Blood Group Substances in Semen and Saliva," presented at the 36th Annual Meeting of The American Academy of Forensic Sciences, Anaheim, CA, Feb. 1984.

[15] Morgan, W. T. J. and Watkins, W. M., "The Product of the Human Blood Group A and B Genes in Individuals Belonging to Group AB," *Nature* (London), Vol. 177, 1956, pp. 521-522.

[16] Watkins, W. M., "Blood-Group Specific Substances," in *Glycoproteins: Their Composition, Structure and Function*, A. Gottschalk, Ed., Elsevier, Amsterdam, 1972, pp. 830-891.

[17] Watkins, W. M. and Morgan, W. T. J., "The A and H Character of the Blood Group Substances Secreted by Persons Belonging to Group A," *Acta Genetica and Statistics in Medicine*, Vol. 6, 1957, pp. 521-576.

[18] Watkins, W. M., "Genetic Regulation of the Structure of Blood Group Specific Glycoproteins," *Biochemistry Society Symposium*, Vol. 40, 1974, pp. 125-146.

[19] Kelder, K., "The Characterization of Saliva and Seminal Stains in the ABH and Lewis Systems Using the Same Stain Extracts," *Canadian Society of Forensic Science Journal*, Vol. 14, No. 4, 1981, pp. 165-171.

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