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The Quantitative Acid Phosphatase Test. A Statistical Analysis of Endogenous and Postcoital Acid Phosphatase Levels in the Vagina

The acid phosphatase test is routinely used in many laboratories as a test for semen in cases involving sexual assault. The basis for this test is the fact that the level of acid phosphatase (ACP) activity is 500 to 1000 times higher in human semen than in other normal body fluids or secretions [*1*]; this ACP is secreted into semen by the prostate gland. It has been amply demonstrated that elevated levels of ACP activity persist in the vaginal pool after sexual intercourse and in semen stains [*2-16*]. Thus the detection of strong ACP activity is considered a fairly reliable indicator of semen.

The test for ACP activity in vaginal pool material collected from sexual assault victims poses several special problems in interpretation. It has been noted by many investigators that low levels of ACP activity are normally present in the vaginal fluids of sexually inactive women [*4,6,7,9,15-17*]; the tissue origin of this endogenous vaginal ACP is unknown. In testing for ACP activity in vaginal pool material (washings or swabs) collected from alleged sexual assault victims, a distinction must be made between normal endogenous ACP levels and "significantly" elevated ACP levels indicating the presence of semen traces. There is no uniform sense as to the proper threshold between normal and elevated ACP levels in vaginal pool material; most laboratories set their own thresholds arbitrarily according to their experience and intuition (see, for example, Refs *4* and *9*). Another area of ambiguity is the relationship between the ACP activity level detected in postcoital vaginal pool material and the postcoital interval. Published studies show clearly that the level of ACP activity recovered from the vagina declines after intercourse. The decline, however, appears to be quite variable [*2,4,9*]; the ACP activity level may remain elevated for as long as three or four days after intercourse or may drop to negligible levels within a few hours. This variation would seem to make questionable any definitive estimation of postcoital time intervals. Moreover, in those cases where the drop-off in activity level is rapid, the ACP test result may be interpreted as negative; this points out that a negative ACP test by no means contraindicates recent sexual intercourse. The frequency of such false negatives is not known although it is a question of obvious medicolegal concern.

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From the foregoing discussion it is apparent that despite much study we yet have something to learn about the interpretation of the ACP test. In particular, our knowledge is still incomplete regarding (1) the range and distribution of ACP activity levels normally found in the vagina, and (2) the range and distribution of ACP levels found in the vagina after intercourse. The question of the distribution of endogenous and postcoital ACP values in the vagina is of particular importance. If these ACP values are distributed according to a standard statistical distribution, for example the Gaussian normal distribution, then it becomes possible to state with good accuracy what proportion of ACP values fall above or below any given value. Such statistical characterization of the endogenous vaginal ACP would allow the distinction of elevated values at defined levels of significance. Similarly, statistical characterization of postcoital ACP distributions might expose any regularities in the seemingly irregular decline of activity after intercourse.

This report presents an analysis of a large collection of data with the objective of defining the ranges and distributions of endogenous and postcoital levels of ACP in the vagina. The data on which this analysis is based were taken from four published studies dealing with the quantitative ACP test and from hitherto unpublished work from this laboratory. Statistical analysis of the data shows that both endogenous and postcoital levels of ACP in the vagina follow predictable distributions. Moreover, it appears that there are in fact regularities in the decline of postcoital levels of ACP activity that allow, within limits, estimates of postcoital intervals. These results thus provide some guidelines for the interpretation of the quantitative ACP test.

Materials and Methods

Analytical Studies

Three series of vaginal ACP determinations were performed in this laboratory. In Series A, the intention was to investigate variation in endogenous and postcoital levels of vaginal ACP in a single individual. Vaginal washings were collected on a daily basis from the volunteer subject throughout one menstrual cycle by the staff at an obstetrics-gynecology clinic at a local hospital as part of an ongoing study by that unit of cyclic variation in hormone levels. Each morning, the subject's vaginal cavity was washed three times with approximately 3-ml volumes of sterile saline; the washings were removed by aspiration into a syringe and transferred to a test tube. About 10 ml of wash was collected each day; the washes were frozen immediately after collection and were stored at −20°C until analysis. At the time of analysis each washing was thawed, centrifuged to remove particulate debris, and assayed for ACP activity. Of the 25 samples collected, 12 were postcoital with an interval between intercourse and collection of 9 to 13 h. Because of the thoroughness of the washing procedure, the remaining samples can be considered representative of the vaginal pool.

Series B was also designed to study individual variation in vaginal ACP levels. Three normal volunteer subjects abstained from sexual intercourse through one menstrual cycle. Vaginal fluids were collected on tampons inserted for a constant time interval, usually on alternate nights, through the cycle. Upon removal from the vagina, tampons were placed in a jar containing 10 ml sterile saline (which was rapidly absorbed by the tampon) and were frozen in the jar until analysis. At the time of analysis, the tampons were thawed and wrung out into a test tube; the recovery of fluid was about 7 ml and, as indicated in control studies, represents about a 1:10 dilution of the vaginal vault fluids. Particulate debris was centrifuged out and the supernatant liquid was assayed for ACP activity. Although this method is not appropriate for sample collection in rape cases, it is very convenient for routine collection of vaginal fluids for chemical or biochemical analysis.

In Series C the intent was to gather additional data on levels of endogenous vaginal

ACP in female population. Vaginal swabs from 19 volunteer subjects, all of whom had abstained from intercourse for five days or longer, were collected and air-dried. At the time of analysis, the swabs were extracted into 0.3 ml sterile saline and assayed.

An additional set of determinations was done on 42 semen samples to establish semen baseline ACP levels. The semen samples were obtained from 30 volunteer subjects who had collected their own samples by masturbation into sterile containers. The semen was allowed to liquify at room temperature and the sperm fraction was separated from the seminal plasma fraction by centrifugation. The clarified seminal plasma was stored at −20°C until the time of ACP assay. It was found that seminal plasma samples could be stored up to several years without significant loss in ACP activity.

The ACP assay in each of these studies followed a standard procedure employing *p*-nitrophenyl phosphate as substrate. Aliquots of sample were assayed at 37°C in a reaction volume of 1.5 ml containing substrate at a concentration of 2 m*M* in 0.1*M* sodium acetate buffer, pH 5.5. The reaction was terminated by the addition of 1.75 ml 1*M* sodium hydroxide and the concentration of the reaction product, *p*-nitrophenol, was measured spectrophotometrically at 410 nm (molar extinction coefficient $\epsilon = 16\ 200$ litres/mole·cm). The amount of sample assayed and the assay time were adjusted such that the change in absorbance was no greater than 1.0 to keep within the linear range of this assay. Enzyme activities are reported in International Units where one unit is defined as the amount of enzyme that turns over 1 μmole of substrate per minute.

Other Sources of Data

Published data from four additional studies were used to extend the statistical base of the analysis. The data from these studies were for the most part used directly as reported; in a few cases there was a question regarding subject history or pathology and these data points were not used. Pertinent details of these studies are outlined below.

Godwin and Seitz [*6*] collected samples as vaginal swabs that were dried, extracted into 1.5 ml saline, and assayed employing naphthyl phosphate substrate. Their study contains data on normal vaginal pool levels for 19 individuals and on postcoital levels for 23 individuals.

Willott [*15*] collected samples on vaginal swabs and assayed them using *p*-nitrophenyl phosphate as substrate. The data set includes 40 determinations of normal vaginal pool levels from five individuals; one of these determinations was very much higher than the rest of the values in the data set and was considered separately from the other 39 values. There were also data on 21 postcoital acid phosphatase determinations.

Gomez et al [*7*] collected samples as 2-ml vaginal washings which were assayed immediately using two different substrates, naphthyl phosphate and thymolphthalein monophosphate. The data set includes 21 determinations considered to represent normal vaginal pool levels and 12 postcoital determinations.

Findley [*4*] collected samples on vaginal swabs which were stored frozen; for analysis, the swabs were extracted in 2 ml saline and assayed using thymolphthalein monophosphate as substrate. The data set includes 32 determinations of normal vaginal pool levels and 108 postcoital determinations.

Results

Distribution of Endogenous and Postcoital ACP Levels in a Single Individual

Table 1 shows ACP levels in 25 vaginal washings collected from a single individual over a menstrual cycle (Series A data): 12 of the washings were postcoital with a postcoital interval of 9 to 13 h and the remainder were considered to be a sampling of the endogenous

TABLE 1—*Acid phosphatase levels in vaginal washings from a single individual.*

Endogenous Vaginal ACP		Postcoital (9 to 13 h)	
0.112[a]	0.250	0.162	8.94
0.122	0.344	0.338	10.2
0.124	0.396	2.91	11.04
0.150	0.458	5.52	12.16
0.218	0.682	7.10	16.48
0.232	1.608	8.32	32.40
0.246			

[a] Values are units per total vaginal wash. Units are defined under Methods and Materials.

vaginal fluids without semen contamination. The use of a single subject, of a thorough vaginal fluid collection procedure, and of a highly reproducible enzyme assay minimized sources of variation in the determination of ACP levels. The principal remaining source of variation was within-individual variation and, as the data in Table 1 show, this variation was considerable. The mean value for the endogenous ACP data is 0.38 units per washing with a standard deviation of 0.40 units; the corresponding values for the postcoital data are 9.63 ± 8.64 units. For both data sets, the range of values spans more than an order of magnitude and in both the high value is more than 2½ standard deviations higher than the mean value. Although the extent of the variation seems larger than one might expect for determinations performed on a single individual, the data in Table 1 should provide a fairly accurate picture of total vaginal ACP levels of the subject individual for the reasons cited above.

To determine a meaningful threshold value to distinguish endogenous vaginal ACP levels from "significantly" elevated postcoital levels, it is necessary to know the distribution of the endogenous ACP levels. If, for example, the endogenous ACP were distributed according to a Gaussian normal distribution, then a reasonable significance threshold level would be a value three standard deviations above the mean (that is, at 1.58 units); only about one in a thousand endogenous ACP values would be expected to exceed this threshold value. Referring to the data in Table 1, ten of the twelve postcoital values would be recognized as significantly elevated if this threshold value were used. (That two postcoital values fall below the threshold value is not particularly surprising given the postcoital interval.) However, one endogenous ACP value also would be considered elevated. A value this high was clearly not expected given a normal distribution of values and this deviation from expectation raised the question of whether endogenous ACP levels are normally distributed.

As a preliminary test to determine whether the endogenous ACP data were distributed normally, the values were ranked and plotted according to their cumulative frequency on normal probability paper (Fig. 1*a*). If the values were normally distributed, the resulting plot should approximate a straight line. However, the plot obtained clearly curves upward on the right; it is not possible to fit a straight line to the points on the graph. This finding suggested that endogenous vaginal ACP levels, at least as represented in this data set, were not normally distributed.

Consideration was given to the possibility that the apparent deviation from normality might be because the distribution of determined values is truncated at the zero value. In such cases, the apparent sample mean is a value higher than the true distribution mean and the calculated sample standard deviation is an underestimate of the true standard deviation. To test this possibility, estimations for means and variances corrected for truncation were derived essentially as described by Selvin [*18*]. Graphical analysis of each

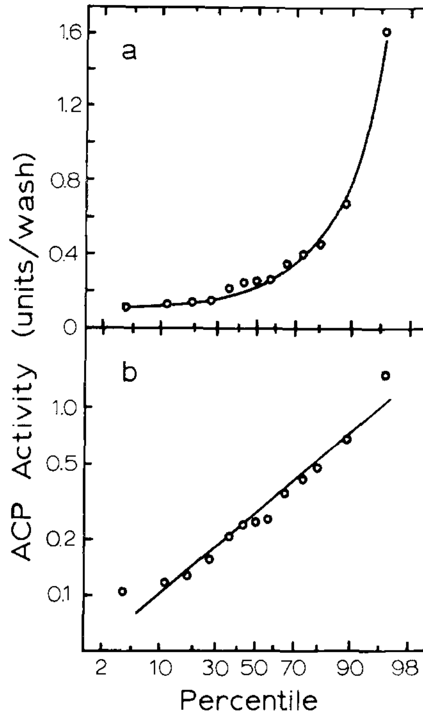

FIG. 1—*Cumulative frequency distributions of endogenous vaginal ACP levels in a single individual. The data from Table 1 have been plotted on* (a) *normal probability coordinates and* (b) *lognormal probability coordinates. Each of the 13 values represents 7.7% of the total data set. Each value can then be represented on the cumulative plot as a step 7.7 percentage points wide; in this illustration, each value is plotted at the midpoint of its step. A straight-line plot indicates a fit to the distribution.*

data set after correcting for truncation showed no significant improvement in the fit to normality.

The endogenous ACP data from Table 1 were then analyzed for fit against several other probability distribution functions. It was found that a lognormal distribution function characterized the data very well. The lognormal distribution differs from the normal distribution in that it is the logarithms of the ACP values rather than the ACP values themselves that are normally distributed. Initial indication of the fit of the data to a lognormal distribution was obtained by plotting the ranked data as before on log probability paper (Fig. 1*b*); the plot obtained is reasonably linear. A more rigorous test of the goodness of fit was provided by using χ^2 analysis to compare the observed distribution of endogenous ACP values with the expected lognormal distribution given the mean and standard deviation parameters calculated from the data [*19*]; the χ^2 test indicated no significant difference at the 5% level. The distributions of endogenous vaginal ACP levels in three other individuals (the Series B experiment) were similarly tested and were found to give reasonable fits to lognormal distributions. It thus appears that the distribution of endogenous vaginal ACP activity levels in single individuals is lognormal.

The postcoital data from Table 1 were also analyzed as described above for the endogenous ACP data. Again it was found that the distribution of ACP levels was best described by a lognormal distribution. The finding that postcoital ACP levels could be

defined by a statistical distribution function indicated the possibility that the postcoital decline of ACP activity could be analyzed in a more rigorous way than heretofore possible; this issue is dealt with in a later section.

Distribution of Endogenous Vaginal ACP Levels in the Female Population

Based on the preceding analysis, it would be expected that endogenous vaginal ACP levels sampled from a random female population might also be distributed lognormally. To test whether this was the case, five independent sets of data were analyzed for fit to lognormal distributions; in addition, for the sake of completeness, each of the five data sets was also tested for fit against normal distributions. Four of the data sets were taken from published studies [*4, 6, 7, 15*]. The fifth data set was based on analyses done in this laboratory (Series C determinations); this data set is presented in Table 2. The mean, standard deviation, and range of values for each of the five data sets are summarized in Table 3. In each case, the cumulative distribution plots on probability paper and on log probability paper provided indication that the data were lognormally distributed. The goodness of fit of each data set to a lognormal distribution was further assessed by χ^2 analysis, which in every case showed no statistically significant differences between the observed and expected distribution of data points (all $P > 0.05$). Thus analysis of the data from these five independent studies, each employing different sampling procedures and analytical methods, demonstrates that endogenous vaginal ACP levels in a random female population are distributed according to a lognormal distribution.

Standardization and Pooling of Endogenous Vaginal ACP Data

In each of the five population studies, the data represent measurements of a single variable, the level of ACP activity endogenous to the vagina. The measurement values differ in units and in scale because different methods and procedures were employed in each study; this is reflected in the summary information on the five data sets presented in Table 3. However, because the same variable was measured in each case, it should be possible to standardize the data in each data set so that the unit and scale differences are factored out. The standardized data from the different studies could then be compared directly by using appropriate statistical tests; if the distribution parameters of the standardized data sets prove to be identical, then it would be legitimate to pool the standardized data from the five studies. If the data could be pooled, it would greatly enlarge the data base and would allow more precise estimation of the vaginal ACP distribution parameters.

TABLE 2—*Endogenous acid phosphatase activity on vaginal swabs from 19 individuals.*

0.0024[a]	0.0182
0.0036	0.0220
0.0041	0.0230
0.0041	0.0292
0.0049	0.0347
0.0082	0.0438
0.0083	0.0444
0.0102	0.0469
0.0116	0.1152
0.0122	

[a] Values are units per swab.

TABLE 3—*Summary of data on endogenous vaginal ACP levels in five studies.*

Study[a]	*n*	Mean	SD	Range	Units[b]—Substrate—Sample
This study (Table 2)	19	0.0235	0.0267	0.0024-0.1152	I.U. *p*-nitrophenyl phosphate per swab
Findley [4]	32	25.75	20.27	3-98.4	I.U. thymolphthalein phosphate per litre swab extract
Godwin and Seitz [6]	19	27.34	24.74	1-85	units α-naphthyl phosphate per swab
Gomez et al [7]	21	22.01	20.95	0.5-90	I.U. thymolphthalein phosphate per litre vaginal wash
Willott [15][c]	39	1.04	1.03	0.08-4.44	units *p*-nitrophenyl phosphate per swab

[a] Further information about each study is given in Methods and Materials.
[b] International units (I.U.) are defined as μmoles substrate hydrolyzed per minute.
[c] One data point from the Willott study is not included in this table; that data point is discussed in the text.

Standardization of the data was achieved by dividing each value in a data set by the sample mean for that data set; the resulting value is unitless and will be referred to as a standardized acid phosphatase (SACP) value. Taking the data in Table 2 as an example, each of the 19 values in the table was divided by the sample mean (0.0235 units/swab); the result is a standardized data set containing 19 SACP values ranging from 0.102 (0.0024/0.0235) to 4.902 (0.1152/0.0235). The mean for each set of standardized data is by definition equal to 1.0. Because standardization involves dividing by a constant value, the SACP values in each data set remain lognormally distributed and the standard deviation of the lognormal distribution is unchanged. Table 4 summarizes the relevant distribution parameters for the standardized data from the five data sets.

If the SACP values from the five different data sets were commonly distributed, then the standard deviations of the distributions of SACP values in each data set should be approximately equal. Table 4 shows this is the case both for the SACP and for the log SACP data. The standard deviations of the log SACP values were tested for equivalence by using Bartlett's test [*19*]; the differences were not significant at the 5% level. The distributions of the five SACP data sets were also compared by a nonparametric ranking test, the H test [*19*]; this tests the randomness of the integration of values from different data sets when pooled. The distributions were not found to differ significantly by this test either ($P > 0.1$). These statistical tests indicate that the differences in the distribution parameters of the five SACP data sets were not significantly different; accordingly, the pooling of the data from the five sets was allowable.

Distribution Parameters and Significance Thresholds for Endogenous SACP in the Vagina

The distribution of the pooled SACP data points is illustrated in Fig. 2. The 130 data points are clearly not normally distributed, there being a significant tail to the right. When each of the five sets of standardized data is plotted cumulatively on log probability coordinates (Fig. 3*a*), almost all the data points fall within a narrow band; only a few points representing low vaginal ACP levels fall outside of this band. A cumulative plot of the pooled data is shown in Fig. 3*b*. The plot is basically linear from about 20% probability up; only the few data points representing low vaginal ACP values fall below this line. The goodness of fit of the pooled standardized data to the lognormal distribution was tested with the result shown in Table 5; the χ^2 test indicates that the fit is quite satisfactory. The lognormal distribution mean and standard deviation calculated from the pooled data therefore provide good estimates of the distribution parameters of endogenous SACP levels in the vagina.

Significance thresholds for the right-hand tail of the standardized vaginal ACP distribution were calculated from the lognormal distribution parameters and are presented in Table 6. The 99% threshold is an SACP value of 6.61; we would expect only 1% of endogenous vaginal SACP values to exceed this threshold value. In fact, none of the

TABLE 4—*Distribution parameters of standardized vaginal ACP data.*

	Standardized Values			Log Standardized Values	
Study[a]	Mean	SD	Range	Mean	SD
This study	1.0	1.14	0.102-4.902	−0.222	0.460
Findley [*4*]	1.0	0.81	0.117-4.311	−0.116	0.345
Godwin and Seitz [*6*]	1.0	0.90	0.037-3.106	−0.257	0.565
Gomez et al [*7*]	1.0	0.95	0.023-4.089	−0.217	0.520
Willott [*15*]	1.0	0.99	0.077-4.269	−0.156	0.360

[a] Further information about each study is given in Table 3 and in Methods and Materials.

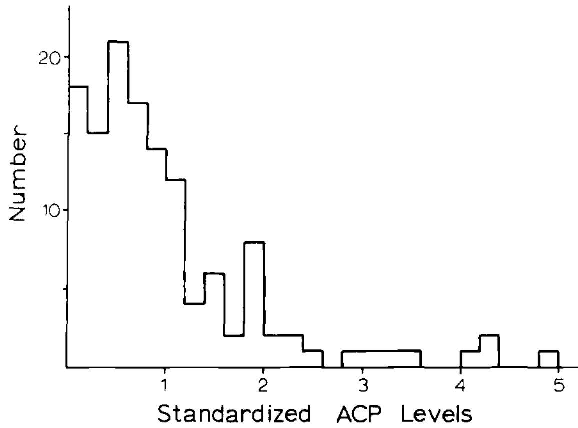

FIG. 2—*Distribution of standardized endogenous vaginal ACP levels. The distribution represents 130 values from five independent studies.*

130 SACP values in the data set did exceed this threshold value. In general, the comparison of observed and expected SACP values exceeding each threshold shows that the predicted values tend to be slight overestimates.

One data point in Willott's study [*15*] was about six times higher than the next highest value in the data set and for this reason was excluded from the distribution analysis up to this point. This anomalous value when standardized is 26.54, which is almost four standard deviations above the distribution mean. A value this high could occur by chance; however, the probability of a chance occurrence would be less than once in 10 000 cases. Alternatively, the value might be a result of a measurement error or an unrecognized

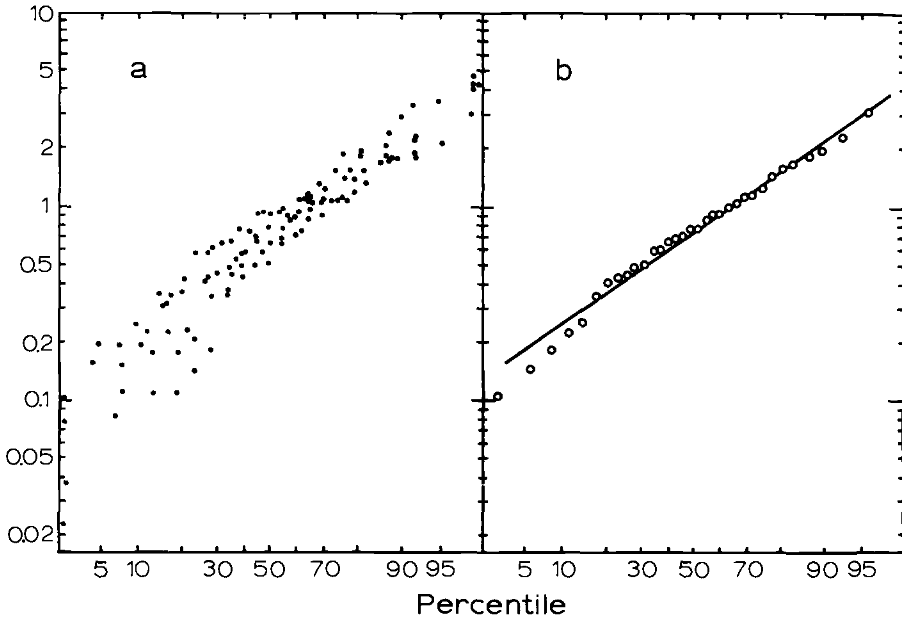

FIG. 3—*Cumulative frequency distribution of standardized vaginal ACP levels. In* (a) *the data from each of the five studies have been plotted independently. The plot in* (b) *represents the pooled data; every fourth point in the pooled data set has been plotted.*

TABLE 5—*Comparison of observed and expected distributions of pooled standardized vaginal ACP levels.*

Standard Deviation Range	SACP Range[a]	Observed	Expected	χ^2
Below −2.0	0.000-0.091	4	2.9	0.42
−2.0 to −1.5	0.091-0.149	6	5.7	0.02
−1.5 to −1.0	0.149-0.245	12	11.9	0
−1.0 to −0.5	0.245-0.402	11	19.5	3.71
−0.5 to 0	0.402-0.660	28	25.0	0.36
0 to 0.5	0.660-1.084	28	25.0	0.36
0.5 to 1.0	1.084-1.781	20	19.5	0.01
1.0 to 1.5	1.781-2.925	14	11.9	0.37
1.5 to 2.0	2.925-4.804	6	5.7	0.01
2.0 to 3.0	4.804-12.957	1	2.9	1.24
Above 3.0	12.957+	0	...	...
Totals		130	130	6.5
For nine degrees of freedom, $\chi^2(0.25) < 6.5 < \chi^2(0.50)$				

[a] The expected distribution was calculated by assuming a lognormal distribution with a mean of −0.180 and standard deviation of 0.431.

vaginal pathology. In any case, this value would by any standard be considered significantly elevated over the endogenous pool levels. This situation indicates the desirability of having an independent method for the identification of semen traces.

Individual Variation in Endogenous Vaginal ACP Levels

The Series A and B studies demonstrated the variation and distribution of endogenous vaginal ACP levels in single individuals. As noted, each of the four individual sets of values was lognormally distributed; the standard deviations of the log-transformed data were 0.331 (Series A data) and 0.079, 0.092, and 0.178 (Series B data). There thus appears to be significant variability in this parameter from individual to individual. Comparison of these standard deviation values with the standard deviation of the pooled population data (SD = 0.431) indicates the intraindividual variation contribution to the total population variation.

The samples in the Series A and B experiments were collected sequentially through the menstrual cycles of four individuals. No evidence was found indicating that the time of

TABLE 6—*Significance thresholds for the standardized vaginal ACP distribution.*

SACP Threshold Value[a]	Proportion of SACP Values Exceeding Threshold Value[b]	Expected	Observed
1.291	0.25	32.5	31
2.356	0.10	13.0	9
3.381	0.05	6.5	5
4.621	0.025	3.3	1
6.606	0.010	1.3	0
8.466	0.005	0.7	0
14.184	0.001	0.1	0

[a] Threshold values were calculated by assuming a lognormal distribution with a mean of −0.180 and a standard deviation of 0.431.
[b] This is equivalent to the probability that a random SACP value will exceed the threshold.

cycle contributed significantly to the individual variability in ACP levels. In the Series A data, which represented vaginal ACP levels in a single individual, there appeared a tendency for the values to be higher after midcycle; this tendency, however, was not statistically significant and no such tendency was seen in the Series B data, involving three additional individuals. The influence of the time of cycle does not seem to have been carefully investigated in any existing study and further work is required before any definitive statement can be made on this question.

Distribution of Postcoital ACP Levels in the Vagina

The analysis of the Series A postcoital data (Table 1) indicated that the distribution of ACP levels was adequately described by a lognormal distribution function. To determine whether other postcoital data could be characterized in terms of this distribution function, postcoital ACP data from the four published studies were analyzed. The postcoital data were partitioned according to postcoital interval: 0 to 3 h, 3 to 6 h, 6 to 9 h, 9 to 12 h, 12 to 18 h, 18 to 24 h, 24 to 36 h, 36 to 48 h, 48 to 72 h, and 72 to 96 h. The intervals ranged in size from 5 to 34 data points with the first six intervals tending to be the most populated; the total set contained 178 postcoital ACP determinations. Only Findley's study [*4*] had interval data sets that contained more than 10 ACP determinations; these four sets (representing the 0 to 3, 6 to 9, 18 to 24, and 72 to 96 h intervals) were analyzed graphically for fit to normal and lognormal distributions. The result in each case was basically the same as in the analyses of the endogenous ACP and the Series A postcoital data; the plot on normal probability coordinates clearly curved upward at higher ACP levels whereas the plots on log probability coordinates were reasonably linear. Chi-square analyses indicated that the fit of the lognormal distribution to the data was acceptable (all $P > 0.25$). Thus the analysis of these four postcoital data sets confirmed the indication given by the Series A data analysis that the distribution of postcoital ACP values can be reasonably approximated by lognormal distribution parameters; this assumption was made for the subsequent analyses.

As in the analysis of endogenous ACP levels, it was desirable to standardize postcoital ACP values so that data from different studies could be compared directly and, if possible, pooled. The standardization of postcoital ACP values was done by dividing each value from a given study by the mean value for the *endogenous* vaginal ACP determined in that study. Thus, for example, the postcoital values in Series A (Table 1) were standardized by dividing each by 0.38, the mean for the endogenous ACP data in Series A. By standardizing both postcoital and endogenous ACP levels on the endogenous ACP mean, the endogenous ACP mean becomes the standard point of reference; this is a logical reference point since it represents the baseline distribution from which deviations in the form of elevated ACP levels are the issue in question.

The postcoital SACP values from Series A and the four published studies were pooled according to postcoital interval; these data are summarized in Table 7. No statistically significant differences in the distributions of the SACP values making up each interval data pool were indicated by the nonparametric H test (all $P > 0.05$) [*19*]. Graphical analysis of the pooled SACP data for each interval showed that the distribution of values in each case was reasonably lognormal; the acceptability of the fit of the pooled interval data to the distribution was confirmed by χ^2 tests (all $P > 0.05$). The lognormal distribution parameters for the ten postcoital intervals are indicated in Table 7.

Pattern of Acid Phosphatase Decay in the Vagina

The decline in recoverable ACP activity is illustrated in Fig. 4; whole semen and endogenous vaginal ACP levels are included in the figure for reference. The most striking

TABLE 7—*Summary of standardized postcoital ACP data.*

	Postcoital Interval, h									
	0-3	3-6	6-9	9-12	12-18	18-24	24-36	36-48	48-72	72-96
Source of values in each interval										
Table 1	...	...	...	12	...	...	...	...	...	...
Findley [*4*]	24	3	30	4	8	24	...	...	...	17
Godwin and Seitz [*6*]	2	1	...	7	4	1	7	1	...	...
Gomez et al [*7*]	...	3	2	...	2	2	2	...	1	...
Willott [*15*]	...	...	2	1	3	3	2	4	5	1
Total number of values in interval	26	7	34	24	17	30	11	5	6	18
Range of values	10.1-1070	1.16-1181	0.78-246	0.43-85.3	0.23-49.0	0.15-28.5	0.37-57.5	0.48-7.62	0.25-7.75	0.08-6.76
Lognormal distribution parameters										
Mean	2.28	1.57	1.36	0.898	0.734	0.382	0.577	0.338	0.090	−0.167
SD	0.535	1.005	0.757	0.699	0.704	0.512	0.681	0.520	0.581	0.491

feature in this figure is the considerable variation in ACP levels in all postcoital time intervals; the values vary 20- to 100-fold. Despite this variation, there does appear to be a regular pattern in the decline of ACP activity in the vagina after intercourse. Figure 5 shows the postcoital means and standard deviations plotted as a function of the postcoital interval. The plot of the lognormal means (Fig. 5*a*) shows that in the initial few hours after intercourse there is a very rapid drop in the level of recoverable ACP activity; on the average, 80 to 90% of the deposited ACP activity was not recovered in samples of vaginal material collected in the first 3-h interval. The magnitude of this initial rapid loss varied considerably from case to case; as indicated in Table 7 and Fig. 4, the postcoital ACP values in the 0- to 3-h interval span two orders of magnitude, from values in the whole semen range to values indicating more than 99% loss. The cause of the initial rapid loss and its variation is not known; it possibly reflects physical loss of seminal material from the vaginal vault. Following the initial rapid drop in SACP values, the decay plot is fairly linear through the first 10 h after intercourse. This is compatible with a first-order decay process with a half-life of about 2 h. Enzyme inactivation reactions typically are first order and this possibly indicates that this phase of the decay curve reflects enzyme inactivation. By 10 h, the mean level of ACP is about 0.5% that of the ejaculate. After 10 h, the rate of decay becomes more gradual, approaching by 50 h a linear decline with a half-life of about 70 h. At 50 h, the mean ACP level is less than 0.1% that of the ejaculate.

The standard deviation plot (Fig. 5*b*) shows that the postcoital SACP distributions are substantially broader than the SACP distributions in semen or in the vagina. The variance in postcoital SACP levels seems to reach a peak at about 3 to 6 h after intercourse and declines after that, approaching the endogenous vaginal SACP variance by 72 to 96 h. This early increase in postcoital SACP standard deviation probably reflects variability in the factors affecting the initial rapid loss.

The curves in Fig. 5 were used to estimate distribution parameters from which were generated probability distributions of postcoital SACP values; the cumulative distribution probabilities for each of the ten postcoital intervals and for the endogenous vaginal SACP

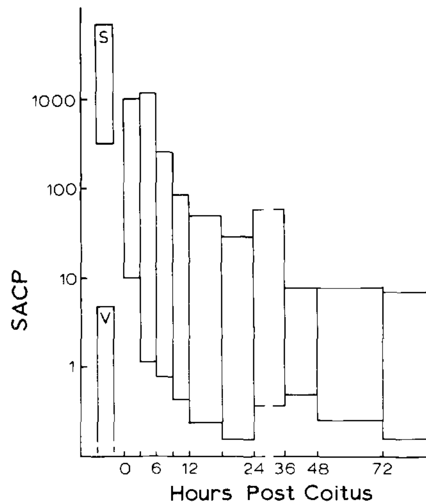

FIG. 4—*Postcoital ACP levels. Each block represents the range of values observed in the indicated postcoital interval; the ranges are listed in Table 7. Included in the figure are blocks representing whole semen* (s) *and endogenous vaginal* (v) *ACP levels; the vaginal block is cut off at the 99% threshold.*

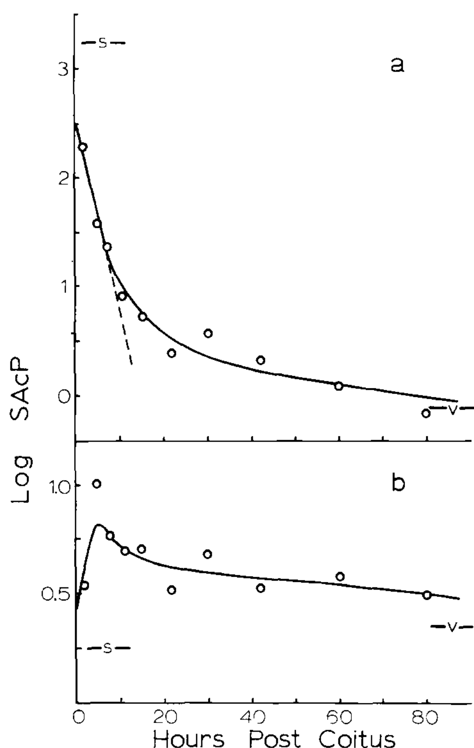

FIG. 5—*Pattern in postcoital decline in vaginal ACP levels. The lognormal mean values* (a) *and standard deviation values* (b) *are plotted as a function of time postcoitus; the values are listed in Table 7.*

are noted in Table 8. The entries in the table are the probabilities that a given SACP value will be equalled or exceeded in any particular postcoital interval. Thus, for example, an SACP value of 256 in a 0- to 3-h interval sample would be equalled or exceeded about 40% of the time. These probability distributions allow two questions to be addressed: the estimation of the postcoital interval and the estimation of the proportion of non-elevated SACP levels in any postcoital interval.

Estimation of Postcoital Interval

The usual approach to the estimation of postcoital intervals has been to set some threshold values which, if exceeded, are taken to indicate intercourse within a defined time period (see, for example, Ref *4*). The probability distributions such as those defined in Table 8 allow such thresholds to be defined in a statistically meaningful way. Threshold postcoital SACP values at the upper 95% level are shown in Table 9; an SACP value equalling or exceeding the 95% threshold value for any postcoital interval would be expected only 1 time in 20. An SACP value of 350, for example, exceeds this threshold at the 6- to 9-h interval and hence would be considered unlikely in this or in any subsequent interval; from this, it might be inferred that intercourse probably occurred within the 0- to 6-h interval. Based on the 178 values in the total postcoital data pool, 8.9 values would be expected to exceed the 95% thresholds; in fact, 4 were observed.

A look at probability distributions listed in Table 8 indicates that a more precise estimation of postcoital interval is not possible. To illustrate, an SACP value between 16

TABLE 8—*Cumulative frequency distribution of standardized postcoital ACP values.*[a]

SACP Value	Postcoital Interval, h										Endogenous Vaginal SACP
	0-3	3-6	6-9	9-12	12-18	18-24	24-36	36-48	48-72	72-96	
1	0.9999	0.983	0.958	0.928	0.881	0.816	0.749	0.663	0.575	0.500	0.348
2	0.9999	0.958	0.908	0.849	0.764	0.663	0.568	0.460	0.358	0.274	0.127
4	0.999	0.911	0.824	0.726	0.603	0.476	0.371	0.264	0.176	0.115	0.029
8	0.995	0.832	0.702	0.568	0.421	0.291	0.203	0.125	0.069	0.034	0.004
16	0.978	0.719	0.551	0.397	0.255	0.152	0.092	0.047	0.021	0.008	...
32	0.925	0.571	0.390	0.242	0.129	0.063	0.032	0.014	0.005	0.001	...
64	0.811	0.417	0.248	0.129	0.056	0.022	0.009	0.003	...	...	...
128	0.626	0.278	0.140	0.059	0.020	0.006	0.002	...	...	...	...
256	0.405	0.166	0.069	0.023	0.006	0.001	...	...	...	...	...
512	0.212	0.087	0.030	0.008	0.002	...	...	...	...	...	...
1034	0.084	0.039	0.011	0.002	...	...	...	...	...	...	...
2048	0.027	0.017	0.004	...	...	...	...	...	...	...	...

[a] Entries are the probabilities that the given SACP values will be equalled or exceeded in the designated postcoital interval.

TABLE 9—*Thresholds for postcoital interval estimation.*

Postcoital Interval, h	Upper 95% Threshold Value
0-3	1446
3-6	857
6-9	342
9-12	148
12-18	70.4
18-24	38.0
24-36	24.4
36-48	15.3
48-72	9.73
72-92	6.64

and 32 would be expected in a 0- to 3-h sample about 5% of the time (that is, the difference between 0.978 and 0.925); the corresponding probabilities for the next six intervals are 15, 16, 15, 12, 9, and 6%. There is not much difference between these probabilities; certainly there is not enough difference to allow an assignment of a postcoital ACP value to a particular postcoital interval.

Overlap Between Postcoital and Endogenous SACP Ranges

It is well recognized that as the postcoital interval increases, an increasing proportion of postcoital ACP determinations yield values in the normal vaginal range [*2,4,6,7,9,15*]. Figure 6 shows the proportion of postcoital SACP values falling below a value of 6.61, the 99.0% threshold for the endogenous vaginal range; the curve, which represents the predicted overlap, matches quite favorably to the observed overlap in each interval. The curve shows that a significant proportion of postcoital ACP values would be expected to fall in the vaginal range even at fairly short postcoital intervals; in the 3- to 6-h interval, the expected overlap is about 15%. The midpoint of the curve is at 14 h; at postcoital intervals exceeding this time, more than half the ACP determinations would be expected to fall in the normal endogenous vaginal ACP range. If different vaginal ACP thresholds are used, the midpoint is altered; at the 95% and 99.9% thresholds the midpoints are 22 and 9 h, respectively.

Discussion

The preceding analysis was undertaken in an attempt to provide answers to several key questions related to the quantitative ACP test and its interpretation: (1) the distribution of ACP activity levels endogenous to the vagina, (2) the cutoff point between normal and elevated vaginal ACP levels, (3) the pattern of ACP activity decline in the vagina after intercourse, (4) the estimation of the postcoital interval, and (5) the chance of "false negative" test results. This study differs from previous studies concerned with these questions in two important respects. First, considerable attention was given to defining the distribution of endogenous and postcoital vaginal ACP levels. The finding that both were distributed according to a standard statistical distribution, the lognormal distribution, allowed statistical analyses to be done and it thus became possible to obtain answers to the questions of interest in a fairly rigorous quantitative fashion. Second, data from different studies were compared directly. Previous attempts to compare such data have failed primarily because different investigators have used different procedures for the collection of samples and for the assay of ACP activity; as a consequence their data have different unit values and cannot be compared directly. In this investigation it was

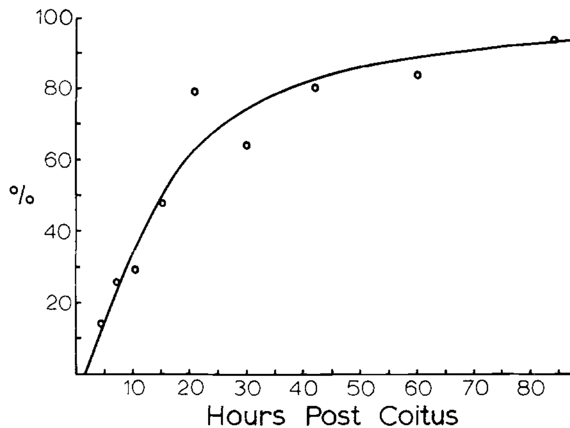

FIG. 6—*Proportion of postcoital ACP values falling in the endogenous vaginal range. The points indicate the proportion of SACP values in each postcoital interval falling below an SACP value of 6.61, the 99% threshold for the endogenous vaginal acid phosphatase range. The curve indicates the predicted proportion of SACP value falling below that threshold value given the postcoital distributions defined in Table 8.*

found that data from independent sources could be standardized against a common reference point and compared directly. The important finding was that the data from different sources showed no statistically significant differences. This allowed pooling of data, which greatly expanded the data base and made possible a more definitive statistical analysis. Moreover, the demonstration that data from different sources are internally consistent lends weight to the general validity of the conclusions reached in this study.

The information contained in Tables 5 through 9 provides basic guidelines for the interpretation of the quantitative ACP test, that is, information on the distribution of endogenous and postcoital ACP levels in the vagina. This information is presented in terms of SACP values, and to make use of this information working laboratories will need to interconvert their own unit values and SACP values. As demonstrated in the Results section, this interconversion is a very straightforward matter. The first step is to determine endogenous vaginal ACP levels in a representative population of females; a minimum of 15 individual samples should be adequate. The lognormal distribution of the determined values can be verified by plotting the values cumulatively on log probability paper; a reasonably good straight-line plot should be obtained. The numerical mean of these values is the standard reference value; it is equivalent to the 1.0 SACP value. The threshold value for distinguishing endogenous and elevated vaginal ACP levels at the 99.0% significance level is 6.606 times the standard reference value (see Table 6 for SACP threshold values).

To illustrate by example, the mean value in Findley's study [*4*] was 25.75 units/litre swab extract (Table 3); the 99.0% threshold in Findley's unit system would be 6.606 times that, 170.1 units/litre extract. If one wished to be more conservative and use the 99.9% threshold value, the corresponding value in Findley's unit system would be 14.184 times 25.75, or 365 units/litre swab extract. Similarly, in the estimation of postcoital interval limits, a value of 37 235 units/litre extract (25.75 times 1446, Table 9) would be the cutoff value for inferring intercourse within 3 h. (It is interesting to note that Findley set his normal-elevated and within-3-h thresholds at 300 and 15 000, respectively, on the basis of experience and intuition.) The valid use of the standardization procedure presumes that the working laboratory employs a single set of procedures for the collection and processing of vaginal samples and for the measurement of ACP activity. If a laboratory receives different types of vaginal samples (for example, swabs from one source and

washings from another source) it would be wise to determine standard reference values for each type separately. Similarly, if collection, processing, or assay procedures are changed, the standardization scale must be recalibrated.

As demonstrated in Table 6, the higher the normal-elevated threshold value is set, the less likely is a stray high endogenous value exceeding the threshold; that is, the higher the threshold values, the less likely a false positive would be observed. Conversely, the higher the normal-elevated threshold value, the greater the proportion of postcoital ACP values falling in the normal endogenous range; that is, the higher the threshold, the greater the proportion of false negatives. Thus, in setting a normal-elevated threshold, these two considerations must be balanced and an appropriate compromise achieved. With the information contained in Tables 5, 6, and 8, it is possible to estimate fairly precisely what proportions of false positives and false negatives would be expected with any set threshold value; such quantitative precision has not been previously possible.

The question of false negatives is of special concern. It has been recognized that as the postcoital interval lengthens, the number of noninformative ACP tests increases. From the postcoital ACP distributions delineated in Table 8, it is apparent that a significant number of false negative results are to be expected even at fairly short postcoital intervals; this was illustrated for the 99.0% endogenous vaginal threshold in Fig. 6. If the more conservative 99.9% threshold is used, the expected proportion of false negatives in the early postcoital intervals is even higher: almost 2% in the 0- to 3-h interval and 26% in the 3- to 6-h interval. Given that the delay between a sexual assault and the medical examination is not uncommonly 3 to 6 h or more, an appreciable proportion of false negatives is thus to be expected. As a consequence, negative ACP test results, even in cases where the postcoital interval is short, should not be unduly emphasized in the laboratory interpretation or in subsequent legal proceedings.

Apart from quantitative guidelines, the analysis of the postcoital data has provided some insight into the fate of seminal ACP in the vagina after intercourse; up to now, there has been no clear picture of this. The decline in recoverable ACP activity appears to involve three distinct phases. In the first phase, there is an almost immediate and usually quite appreciable loss of recoverable ACP. The extent of this initial rapid loss is quite variable, suggesting that it is due to physical processes such as semen drainage from the vagina, dispersion of seminal material in the vaginal cavity, or dilution of the seminal material with vaginal secretions. Physical processes such as these were cited by Davies and Wilson [*2*] as a possible explanation for some of the variability in their data. Additional support for this interpretation comes from quantitative studies on sperm recovery after intercourse; of the 300 million sperm in the average ejaculate, rarely are more than a few percent ever recovered in vaginal washings, even when samples are collected shortly after intercourse [*20,21*]. The second stage is characterized by a first order decay of recoverable ACP activity and occurs in the first 10 to 12 h postcoitus. This pattern of decay is typical of heat-mediated or chemically mediated enzyme inactivation processes, and it is probable that this phase of decay involves such a process. The enzyme half-life during this phase is about 2 h (that is, the enzyme activity decreases by half every 2 h). In the final phase, ACP activity is lost at a much more gradual rate; the kinetics of activity decay during this phase are also compatible with an enzyme inactivation process. It is not clear what the difference is between the second and third phases. It is plausible that semen contains the seeds of its own destruction; semen is known to undergo self-degradation in vitro [*22*] and similar self-degradative processes may occur in the vagina. The second phase may then represent the facilitated semen self-destruction that gives way as the seminal degradative enzymes themselves decompose to the third phase, in which the decay is mediated by endogeneous vaginal factors.

This three-phase hypothesis is somewhat sketchy and requires further experimental analysis to verify details. Nevertheless, as it stands it provides a general framework within

which to consider the fate of other seminal components of interest such as sperm, the p30 semen marker protein [23], the ABO substances, and the enzyme genetic markers [22]. If the first phase basically involves physical processes, then all seminal components should be affected to an appreciable extent. Given that the ACP data indicate that the loss of activity in the first phase varies considerably, ranging from the almost negligible to 99% plus, this first-stage loss would surely be the most profound factor in determining what analyses could be done on postcoital vaginal material. If the second and third phases primarily involve chemical inactivation processes, then each seminal component should have its own characteristic half-life in each phase. From the available literature on the persistence of seminal components in the vagina, it is not possible to do more than guess about half-lives; accurate interpretation will require more thorough quantitative experimentation than has been done to date. In principle, the second- and third-phase processes can also occur in vaginal swabs or washings after collection, and it is of obvious concern to know how these processes affect the storage life of the various semen components in vaginal samples.

Regardless of the mechanisms of ACP decay in the vagina after intercourse, it is likely that the recoverable levels of the various seminal components will correlate fairly directly to the recoverable level of ACP activity. This suggests that the quantitative ACP test has value beyond its present use as an indicator of the effective semen dilution in test samples. It is known, for example, that given the semen level of the genetic marker enzyme phosphoglucomutase, an effective dilution of 1:20 to 1:40 precludes reliable electrophoretic typing [24]. A quantitative ACP assay of test material would indicate whether a dilution on this order had occurred and would thus indicate the feasibility of typing for this genetic marker. This application of the quantitative ACP test does not appear to have been suggested before; however, preliminary tests of this sort become increasingly important as the analyst must choose which test from an enlarging repertoire of potentially informative tests to apply in particular case situations.

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