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Vaginal Odors: GLC Assay Method for Evaluating Odor Changes

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Abstract □ A GLC assay technique, previously applied to the evaluation of the influence of nitrofurazone on pathological vaginal odors, was refined and tested in a study of vaginal secretion odors in normal women before and after administration of a suppository. The principal methodological refinements included considering changes in the concentrations of seven frequently encountered malodorants and testing results for their statistical validity. The technique was sufficiently sensitive to indicate statistically significant ($p < 0.05$) changes in a group of 10 subjects.

Keyphrases □ Vaginal odors—method and GLC assay technique for evaluating odor changes, suppository induced □ Odors, vaginal—method and GLC assay technique for evaluating odor changes, suppository induced □ GLC—detection of changes in vaginal odors

Established techniques exist to determine the type and the number of microorganisms that may produce odors in the vaginal secretions. However, formation of malodorous compounds *in vivo* may depend also on the secretions of changing composition. Reduction in bacterial or fungal counts *in vitro* or *in vivo* cannot be *a priori* equated with the activity in odor suppression; lack of such correlations in axillary odors was reported (1, 2).

Earlier work showed that a combination of GLC and sensory techniques permits an evaluation of the reduction of infection-induced vaginal odors by nitrofurazone (3, 4), as well as a comparison of the number of malodorous compounds in normal secretions (5). Even such a simple indicator as the number of malodorous compounds was sufficient to assess gross changes.

It was felt that further and more quantitative refinements in the odor evaluation technique were desirable. The degree of the malodorosity of each component should be considered. Changes should be objectively quantified by observing the concentration changes (GLC peak areas) of the same odorant. Finally, spurious and arbitrary interpretations should be avoided by application of some simple statistical tests so that an estimate of the significance of the changes can be obtained.

The usefulness of these refinements was investigated in a study of odor changes following an intravaginal administration of a suppository.

EXPERIMENTAL

Sampling—Samples of vaginal secretions were obtained using heat-sterilized and deodorized Teflon inserts (Fig. 1). The insert carried 480 circular smooth-edged pits, each 0.15 cm (0.06 in.) wide and 0.07 cm (0.03 in.) deep. A Teflon slip-ring collar allowed adjustment of the depth of insertion. The pits and the surface of the insert served as mechanical traps for mucus. After 20 min, the insert was removed, placed in a clean glass container (Fig. 1), and stored in a freezer until analyzed.

Analysis—Inserts, still in their glass containers, were brought to room temperature. A total volume of 6000 ml of high purity ("zero" grade) helium was passed through the vessel at a rate of 100 ml/min. The collection arrangement is shown in Fig. 1. The vapor-saturated helium then entered a vapor collector, containing 5 g of highly purified, high surface area (>300 m^2/g), nonpolar styrene–divinyl copolymer[1], where organic vapors were absorbed but water vapor was retained to a negligible extent. Vapors then were removed from this collector and transferred in one single injection into a gas chromatograph, using procedures and devices described previously (6).

The chromatographic partition column was a 6.09-m (20-ft), 0.31-cm (0.125-in.), stainless steel tubing packed with 2.5% Carbowax 20M on 60–80-mesh Chromosorb G support, acid washed and dimethylsiloxane treated. The carrier gas was high purity helium introduced at 60 ml/min; the temperature was elevated linearly from 60 to 180° at 2°/min. The effluent was split, with one half flowing to a hydrogen flame-ionization detector and the other half flowing to a sniffing port supplied with a controlled flow of air humidified to reduce nose irritation (7).

An analyst, specialized in the sensory evaluation of GLC effluents, characterized the odors of the separated components as

[1] Chromosorb 102, Johns-Manville Celite Division.

Table I—Example of a Computer-Transcribed Odorogram of Vaginal Odors[a]

Peak	Retention Time, min	Peak Area	Percent Peak Area	Odor Note	Hedonic Code	Peak	Retention Time, min	Peak Area	Percent Peak Area	Odor Note	Hedonic Code
1	1.57	−0.00	0.00	—	—	36	23.21	−0.00	0.00	—	—
2	1.72	−0.00	0.00	—	—	37	24.06	8.96	0.13	Aldehydic	P
3	1.85	−0.00	0.00	—	—	38	24.51	−0.00	0.00	—	—
4	2.43	−0.00	0.00	—	—	39	26.10	−0.00	0.00	—	—
5	2.54	−0.00	0.00	—	—	40	26.75	18.56	0.28	Unpleasant	XX
6	2.72	−0.00	0.00	—	—	41	27.04	58.24	0.87	Acidic	XX
7	2.94	1.20	0.02	Pleasant	P	42	27.40	92.16	1.38	Acidic	XX
8	3.19	−0.00	0.00	—	—	43	28.00	−0.00	0.00	—	—
9	3.90	−0.00	0.00	—	—	44	29.25	−0.00	0.00	—	—
10	4.40	14.52	0.22	Yeasty	N	45	30.30	32.64	0.49	Acidic	XX
11	4.75	−0.00	0.00	—	—	46	31.75	788.48	11.83	Sweet	P
12	5.42	3.36	0.09	Fatty	X	47	33.87	3.64	0.06	Sweet	XX
13	5.73	−0.00	0.00	—	—	48	34.46	−0.00	0.00	—	—
14	5.97	−0.00	0.00	—	—	49	36.24	−0.00	0.00	—	—
15	6.46	−0.00	0.00	—	—	50	37.69	896.00	13.45	Fruity	P
16	7.33	−0.00	0.00	—	—	51	39.19	134.40	2.02	Spicy	P
17	8.45	−0.00	0.00	—	—	52	40.40	16.00	0.24	Cheesy	XXX
18	8.78	−0.00	0.00	—	—	53	40.84	14.72	0.22	Cheesy	XXX
19	9.07	−0.00	0.00	—	—	54	41.55	3.84	0.06	Unpleasant	XX
						55	42.31	152.04	2.28	Acidic	XX
20	10.37	26.24	0.39	Unpleasant	XX	56	42.94	102.40	1.54	Fruity	P
21	10.91	−0.00	0.00	—	—	57	43.96	900.00	13.51	Pungent	X
22	11.67	−0.00	0.00	—	—	58	44.69	880.64	13.22	Pungent	X
23	12.37	−0.00	0.00	—	—	59	45.78	409.60	6.15	Spicy	P
24	13.04	−0.00	0.00	—	—	60	46.22	−0.00	0.00	—	—
25	14.12	−0.00	0.00	—	—	61	47.30	−0.00	0.00	—	—
26	14.93	25.60	0.38	Odor	N	62	48.85	950.00	14.26	Bitter	XXX
27	16.43	32.00	0.48	Unpleasant	XX	63	50.10	51.20	0.77	Pleasant	P
28	17.06	−0.00	0.00	—	—	64	50.73	900.20	13.51	Fruity	P
29	17.88	−0.00	0.00	—	—	65	52.01	−0.00	0.00	—	—
30	18.52	2.32	0.03	Fruity	P	66	52.85	17.02	0.27	Unpleasant	XX
31	19.00	55.04	0.83	Bitter	XXX	67	54.15	28.16	0.42	Unpleasant	XX
32	19.48	−0.00	0.00	—	—	68	55.31	−0.00	0.00	—	—
33	20.37	−0.00	0.00	—	—	69	55.81	−0.00	0.00	—	—
34	20.76	−0.00	0.00	—	—	70	59.67	−0.00	0.00	—	—
35	22.06	43.52	0.65	Fatty	X	71	60.06	−0.00	0.00	—	—

[a] Sample number: Subject 10, 1 pm, day before treatment. Total sample size: 666,300 in.^{2}, odorous peaks only. Peak areas of nonodorous peaks are not indicated. The areas are in square inches.

they emerged from the column through the sniffing port, using the method described earlier (3–5, 8). This method was further refined by using, in addition to the odor descriptors, a hedonic category scale: X, XX, and XXX designated unpleasant odors in the order of increasing intensity of unpleasantness; P, PP, and PPP designated pleasant odors in the order of increasing pleasantness; and N was used for odors that were neither primarily unpleasant nor pleasant[2].

In the described arrangement, odorants preconcentrated from a 6000-ml sample of the vapors are evaluated in one single analysis. Therefore, each compound present in the sample is delivered for the sensory assay, even after some subsequent dilution in the GLC column and the sniffing port, at a concentration 50–100 times higher than in the original sample vapor space. Components exhibiting odor do so at an enhanced intensity which facilitates their odor characterization. On the other hand, if a component is not odorous when it emerges from the port, its concentration in the original sample was much below its odor threshold and, therefore, it is not odor relevant.

Schedule—Ten healthy subjects were used[3]. Usual precautions (5) were taken to reduce the possibility of interferences by extraneous factors (cosmetics, spiced foods, *etc.*).

Three samples were taken from each subject at regular intervals on 2 consecutive days. The samples "before" treatment were taken at 9 am, 1 pm, and 5 pm of the "pretreatment" day; the suppository was administered at 7 am on the subsequent or "treatment" day, and samples were again taken at 9 am, 1 pm, and 5 pm. Thus, they represented secretions 2, 6, and 10 hr after treatment. Additionally, 60 samples representing usual (nontreated) vaginal conditions were taken from the same 10 subjects on different days under the same precautions as on the pretreatment day to collect more information on the normal chromatograms.

The vaginal suppository[4] contained 0.0004 g phenylmercuric acetate, 0.0004 g methylbenzethonium chloride, and 0.002 g methylparaben in a water-dispersible base.

Data Reduction—Areas of odorous peaks were measured with an electronic integrator[5], and the accumulated data were transferred to punched cards and printed out in tabular form. A sample analysis is shown in Table I. From the calibration of the instrument with *n*-hexane, peak area multiplied by 5 yields the approximate concentrations of the respective odorants in nanograms per liter of gas space in equilibrium with the sample[6].

RESULTS AND DISCUSSION

The effect of an odor change may be reflected by the GLC–sensory assays in several ways. The number of the odorous or malodorous chemical species may increase or decrease, and their relative malodorosity may change. This may become evident in the odor assay of the effluents at the exit from the GLC partition column or by a more exact procedure of comparing the GLC areas of

[2] Several years of experience with this technique has demonstrated that clearly unpleasant odorants (amines, lower fatty acids, sulfides, mercaptans, *etc.*) are uniformly judged unpleasant by different analysts when presented, without identification, for sensory ratings in a form of GLC effluents. Ratings are more individualistic on odors that are pleasant or neutral. It was shown previously (9) that, even with a minimum of experience, individuals reasonably reliably distinguish three to four levels of odor intensity.

[3] Clinical data on the subjects are available from the authors upon request.

[4] Norforms, Norwich Pharmacal Co.

[5] Hewlett-Packard.

[6] One nanogram of an organic substance gives a GLC peak with an area of 0.2 in.^{2}.

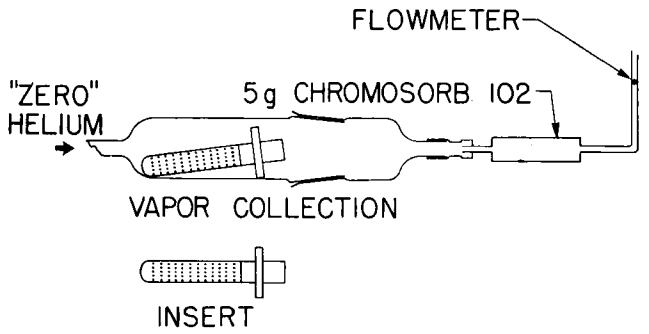

Figure 1—*Vaginal insert (bottom) and the arrangement for transfer of vapors from insert to an organic vapor collector.*

the corresponding peaks in different samples. With the latter procedure, the GLC positions of odorants most commonly occurring in the samples are ascertained first.

Baseline Data—The gas chromatograms of 90 samples taken from 10 subjects on days not influenced by any treatment, including the 30 samples taken on the day before the experimental treatment, served to characterize the GLC profiles of usual secretions, with particular emphasis on malodorous compounds.

An overview on the number and GLC positions of the usually occurring malodorants was obtained from inspection of Fig. 2. This histogram indicates the frequency of occurrence of malodorous chemical species for each minute of retention time. As an example, such a compound was noted at the 8th min in 31 out of 90 analyzed samples. A Kovats index (10) scale (indicated at the top) provides retention values that are largely independent of the flow rates, temperatures, and the amount of stationary phase in the column, as long as the same phase is used.

There were seven distinct retention time points where malodorants frequently appeared (arrows) and seven additional points (stars) with less pronounced frequency-of-occurrence maxima. The seven most frequently occurring compounds had estimated Kovats indexes of 960, 1100, 1300, 1470, 1720, 1750, and 1840.

The following possible indicators derivable from the GLC–sensory assays were compared for their ability to reflect changes in samples from the pretreatment to the treatment day: *(a)* total

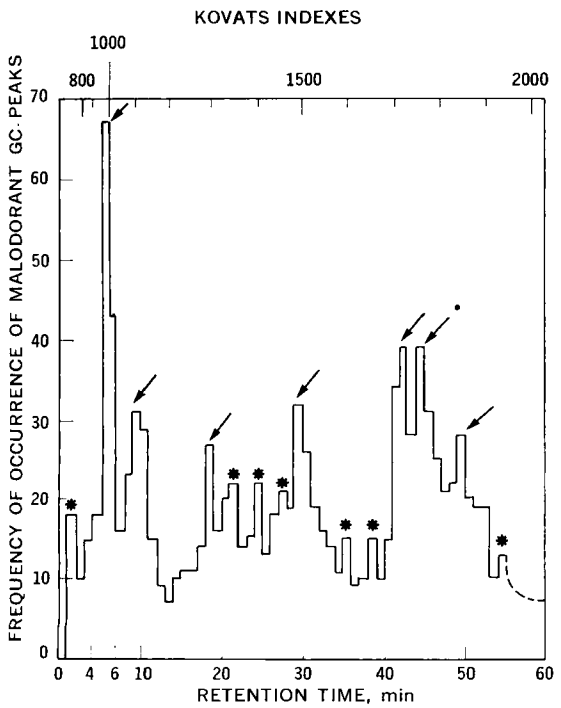

Figure 2—*Histogram of malodorant peak occurrences in gas chromatograms of vaginal vapors (10 subjects, 90 chromatograms, all samples on days not affected by treatment).*

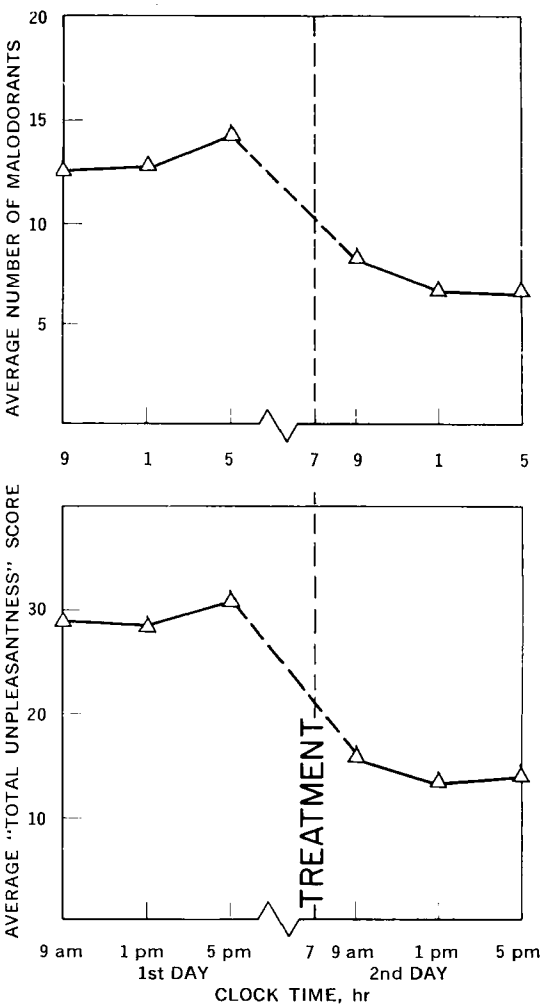

Figure 3—*Change in the number of malodorants and average total malodorosity score during the experiment.*

number of peaks, *(b)* total number of odorous peaks, *(c)* total number of malodorous peaks, *(d)* sum of GLC areas of all odorous peaks, and *(e)* GLC areas of the seven most frequently occurring malodorants.

Total Number of Peaks—This value represents the minimum number of chemical species present in the sample. The number of peaks ranged from 22 to 87, with an average number of 55. This value was not influenced by the treatment to a statistically significant degree (Wilcoxon sum of ranks test).

Total Number of Odorous Peaks—The number of peaks accompanied by a detectable odor ranged from 2 to 38. It also was not significantly influenced by the treatment (Wilcoxon sum of ranks test).

Total Number of Malodorous Peaks—Figure 3 indicates the average number of malodorous peaks per sample for different time points in the experiment. Since each malodorous peak in each gas chromatogram was scored for the degree of malodorosity on an arbitrary three-point scale (X, XX, and XXX), a derived indicator was possible: a cumulative malodorosity score which is

Table II—Sum of Ranks on GLC–Sensory Data[a]

Hours after Treatment	Wilcoxon Sum of Ranks Test, p	
	Number of Malodorants	Malodorosity Score
2	Insignificant	Insignificant
6	<0.05	<0.002
10	<0.05	<0.002

[a] Ten pairs of observations.

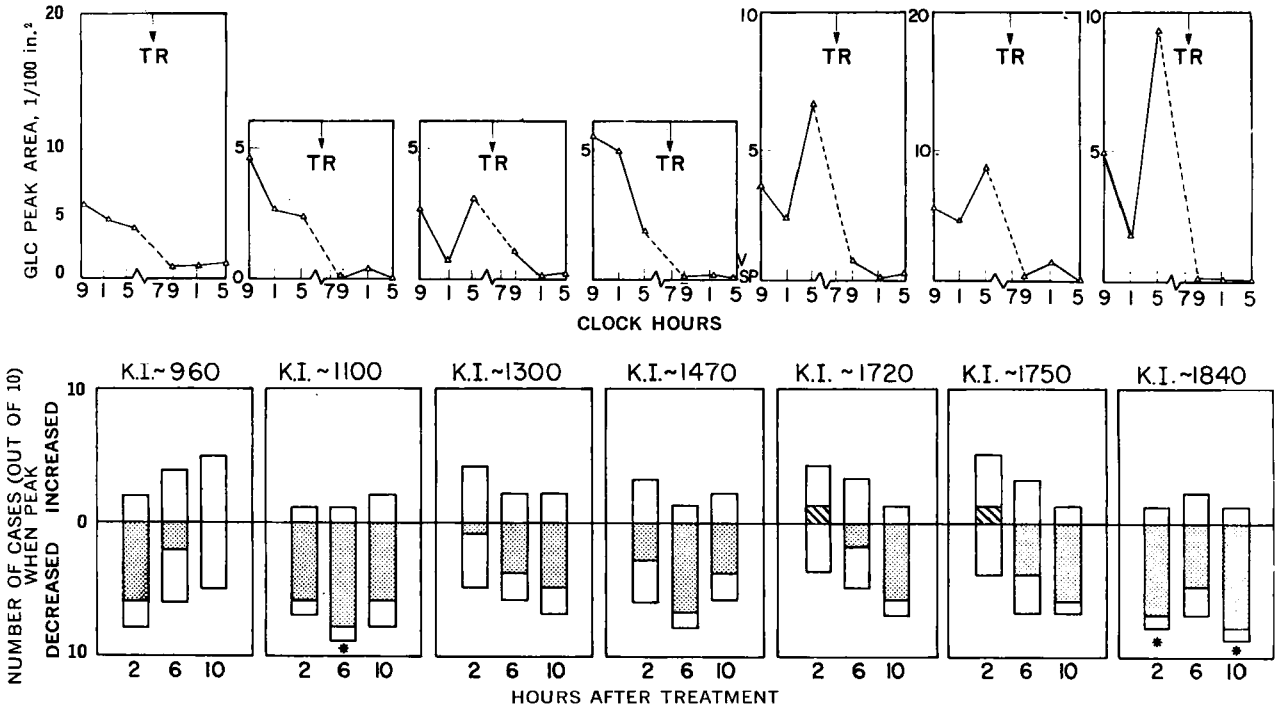

Figure 4—*Effect of suppository on concentrations of seven malodorants most frequently occurring in vapors of vaginal odors of 10 subjects. Kovats indexes of peaks to which each set of diagrams belongs are indicated by K.I. numbers between the two rows of diagrams. The treatment point is indicated by arrows marked TR. Above: median values of peak areas. Below: histogram of frequencies of the direction of peak size changes. Key:* □, *excess of decreases over increases; and* □, *excess of increases over decreases.*

the sum of scores per sample. As an example, it is 38 for the gas chromatogram of Table I. The rationale was that a few high-score peaks may have as much odor significance as a larger number of low-score peaks. The cumulative malodorosity is also plotted in Fig. 3.

The statistical significance of observed changes was estimated using the Wilcoxon sum of ranks test. The 10 samples taken at 9 am on the day before treatment were coranked with 10 samples taken at 9 am of the treatment day, *etc.* (Table II). This procedure was used to minimize the possible effect of daily cyclic activities. The cumulative malodorosity score indicated higher significance levels than the number of malodorous peaks.

Concentration of Odorous Compounds—The average concentration of all odorous compounds, regardless of their odor type, was 2.1×10^{-6} g/liter of sample vapor for samples taken on days not influenced by any treatment. It was not influenced by the treatment to a statistically significant degree.

Concentrations of Seven Malodorants—The upper part of Fig. 4 reflects, separately for each of the seven most frequently occurring malodorants, the changes in median concentration during the experiment. The median value was used to combine the data from the subjects because the peak areas varied from 0 (no peak observed or the peak was nonodorous) to as high as several hundred units. In such a situation, an arithmetic average is inappropriate and a logarithmic average is impossible because of zero values. A median value of zero results when six or more samples from 10 subjects do not exhibit the particular peak.

Table III—Statistical Significance Test on Observed Changes of Concentrations of Seven Malodorants[a]

Hours after Suppository	Lowest Rank Sum (after Suppository)	z Value	Probability that Effect Occurred by Chance Only
2	1204	3.80	<0.002
6	1231	3.41	<0.002
10	1151	4.55	<0.002

[a] Wilcoxon stratified rank test of seven strata, 10 pairs per stratum.

The correlations between odors and concentrations do not necessarily follow a normal statistical distribution. Therefore, nonparametric tests are preferred for evaluation of the statistical significance of the observed changes. The individual data were paired, each pair consisting of one pretreatment sample and the sample taken from the same subject at the same hour of the day of the treatment. This procedure should minimize the effect of daily cyclic activities. The Wilcoxon stratified rank test (11) was used, with seven malodorants serving as seven strata, each containing 10 pairs. This procedure tends to equalize the relative influence of the malodorants, removing the difference in the average concentration levels of the seven malodorants as a factor in the comparison. While some odorants may be more critical than others, the seven malodorants were weighted equally.

Table III presents the statistical results. The change in concentration of the more frequently occurring malodorants jointly reached statistically significant levels.

The lower graphs in Fig. 4 represent a simpler test. The relative direction of change in the peak areas of each malodorant was the only factor considered; decrease upon treatment was plotted downward, and increase was plotted upward. The shaded areas indicate the relative balance of the two directions. The Sign test was used to evaluate the statistical significance of the changes. Data for all seven malodorants were pooled separately for the 9 am samples of the pretreatment and treatment day, *etc.* Table IV

Table IV—Sign Test on Direction of Change in GLC Peak Areas of Seven Malodorants

Hours after Treatment	Total Number of Cases in Which a Change in Peak Areas Occurred	Number of Cases in Which the Peak Area		Statistical Significance Level
		Decreased	Increased	
2	62	42	20	<0.05
6	64	47	17	<0.01
10	63	47	16	<0.01

Table V— Individual Differences in Changes of Concentration of the Seven Most Frequently Occurring Malodorants

GLC Kovats Index of Malodorant	Sampling Time	Number of Cases (out of 10) where Malodorant Content Increased after Treatment									
			Subject Identification								
		Number	1	2	3	4	5	6	7	8	9	10
≈960	9 am	2		x			x					
	1 pm	4			x	x	x	x				
	5 pm	4		x		x	x	x				
≈1100	9 am	1										x
	1 pm	1						x				
	5 pm	2				x						x
≈1300	9 am	3					x			x		x
	1 pm	2							x	x		
	5 pm	2	x							x		
≈1470	9 am	3			x			x				x
	1 pm	1						x				
	5 pm	2			x							x
≈1720	9 am	4					x	x		x	x	
	1 pm	3							x	x	x	
	5 pm	1									x	
≈1750	9 am	5			x		x	x			x	x
	1 pm	3					x		x	x		
	5 pm	1									x	
≈1840	9 am	1						x				
	1 pm	2								x	x	
	5 pm	1										x
		Total[a]	1	2	4	4	7	8	3	7	6	7

[a] Out of 21 comparison pairs per subject.

indicates that this method of data analysis also shows statistically significant changes.

Table V brings out the degree of variability of response in different subjects. Since the response to treatment varied considerably from subject to subject, this variability should be considered in any similar testing study and should have a bearing on the total number of subjects used to reach statistical significance.

CONCLUSIONS

A refined form of GLC–sensory assaying of vaginal odors indicated that approximately 14 malodorants appeared frequently in vapors of the vaginal secretions of 10 normal healthy women. Seven malodorants appeared with considerable frequency, and changes in their concentrations were used to follow the effect of a vaginal treatment.

This refined method may provide a more quantitative basis for evaluating vaginal treatments in terms of malodorant concentration changes.

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