ACKNOWLEDGMENTS AND ADDRESSES

Received October 6, 1969, from *Auburn University, School of Pharmacy, Auburn, AL 36830*

Accepted for publication November 17, 1969.

This work was supported by Contract Number DA-49-193-MD-2985 from the U. S. Army Medical Research and Development Command, and in part by the office of General Research, The University of Georgia, Athens, Georgia.

This is contribution Number 710 from the Army Research Program on Malaria.

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Odor Threshold and Gas-Chromatographic Assays of Vaginal Odors: Changes with Nitrofurazone Treatment

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Abstract □ Although vaginal disorders are frequently accompanied by malodors, the efficiency of drugs to reduce the malodors by reducing the infection has been difficult to estimate. Recently, techniques have become available for combining sensory and gas-chromatographic approaches to compare odor intensities of complex vapor mixtures. These techniques were applied to vaginal odors. An apparatus was devised to collect vaginal vapors in a form suitable for odor-revelant gas-chromatographic analyses, as well as for odor-threshold determinations. Odor changes in vaginal vapors upon treatment with nitrofurazone vaginal suppositories were studied on five patients with several types of disorders (hematuria, endometritis, and stress incontinence). The distribution of odorous components in the gas chromatograms reflected, through disappearance of many malodorous peaks, a significant reduction in the content of the malodorous volatile compounds in the vaginal vapors. Odor-threshold measurements were conducted in an apparatus where nonodorous methane tracer and a hydrogen-flame ionization detector were used to measure the degree of vapor dilution needed to reach the threshold, using the ASTM odor-threshold test design combined with ED_{50} statistics. These measurements similarly indicated that odors were reduced by the drug.

Keyphrases □ Vaginal odor threshold, intensity—analysis □ Odors, vaginal—threshold, intensity determination □ Nitrofurazone effect—vaginal odors □ GLC—analysis

It is usually noted that a reduction in bacterial population in suitable media is accompanied by a reduction in the odors. Odor reduction is often subjectively observed when the antibacterial agent nitrofurazone[1] is used in the treatment of vaginal disorders accompanied by malodor. In the absence of a satisfactory method for quantitative or precise qualitative assay of odors, such observations have remained in the form of testimonials.

Recently, techniques have become available for more detailed studies of the odors in complex mixtures. These techniques are based on combinations of psychophysical (sensory) and gas-chromatographic methods, the latter providing means for collecting and separating the components of odorous vapors. In the present work these techniques were adapted to assay the vaginal odors and their changes upon treatment with an antibacterial preparation. Odor thresholds of vaginal vapors were also determined.

[1] Furacin Vaginal Suppositories, Eaton Laboratories, Division of the Norwich Pharmacal Co.

PRINCIPLES OF ODOR MEASUREMENT

The odor results from interaction of vapors with the observer's chemoreceptors (primarily olfactory and to some extent trigeminal). Substances differ in odor thresholds, *i.e.*, in the lowest concentrations in air at which the odors of their vapors can be detected. Observers also differ in sensitivity (olfactory acuity), so that odor thresholds are represented not by sharp concentration levels but rather by concentration zones, within which the thresholds for different observers can vary severalfold. To a lesser extent, thresholds as estimated by the same observer may vary from time to time.

Above threshold levels, odor intensity increases in proportion to a fractional power (0.2–0.7) of the odorant concentration in air. These relations are expressed in Stevens' law (1, 2):

$$I = k\,(C - C_{\text{thr.}})^x \qquad \text{(Eq. 1)}$$

where I is psychophysical odor intensity, k is a coefficient (small for weak odors, large for strong odors), C is concentration of the odorant in air, $C_{\text{thr.}}$ is threshold concentration of the odorant, and x is an exponent. The values of k, $C_{\text{thr.}}$, and x are not necessarily related. Corollaries of this expression are: (*a*) odor intensity experienced by an observer does less than double when the odorant's concentration is doubled; *e.g.*, for $x = 0.5$, the concentration must increase by a factor of 4 to increase the intensity by a factor of 2; (*b*) if an odorant A has smaller values of k and x than another odorant B, an undiluted A can smell weaker than undiluted B, while diluted A can smell stronger than similarly diluted B; (*c*) since the highest possible concentration of an odorant is at its saturation pressure, odorants with low x, or high threshold and an average x, may never reach very high odor intensity.

Odor-threshold values, therefore, do not necessarily indicate the undiluted odor intensity; rather, the odor of a lower threshold odorant will be noticed farther from the source than will that of a higher threshold odorant. In addition, above the threshold, the character of the odor can be objectionable, as with the malodors, or acceptable, as with fragrances.

The composition of odorous vapors of biochemical origin is complex, with many odorants participating. Odorants present at subthreshold concentrations can summate (sometimes even synergistically) to reach threshold and can modify the character of odors of other substances which are present at suprathreshold levels. In odor assay, therefore, all those substances that are present at levels exceeding reasonable fractions, *e.g.*, one-tenth of their threshold concentrations, must be considered. Gas-chromatographic techniques permit the delivery of separated components for sensory

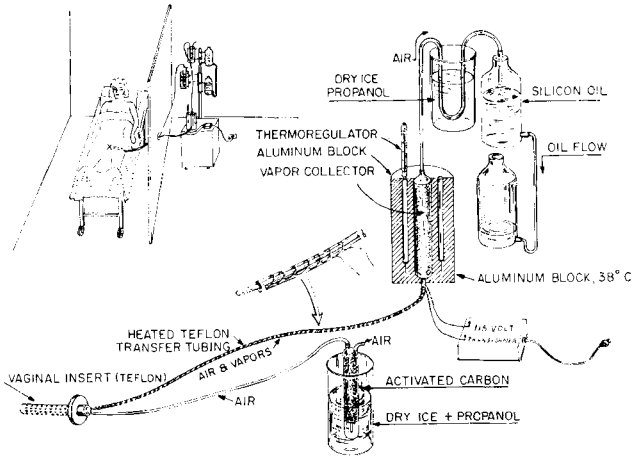

Figure 1—*Apparatus for collection of vaginal vapors.*

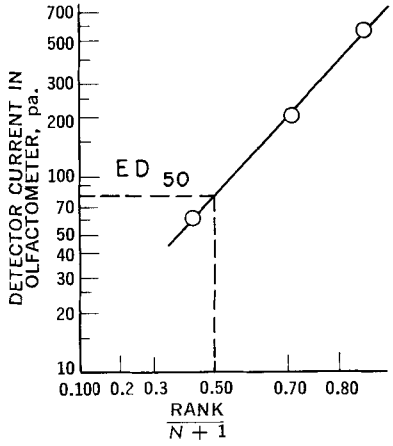

Figure 2—*Example of ED_{50} odor-threshold determination of vaginal vapors.*

assay at concentrations 10–20 times higher than those in the original odorous air mixtures. In this way, mixtures can be studied for predominance and intensities of certain odor notes (*e.g.*, obviously objectionable, fragrant, *etc.*).

METHODS

Vaginal Vapor Collection—Figure 1 shows the arrangement for vaginal vapor collection. A perforated Teflon tube 1.91-cm. (0.75-in.) i.d. and 9.52 cm. (3.75 in.) long (with the disklike collar) was provided with two openings at the outer end. One of these was connected to a vapor collector by Teflon tubing heated along its entire length by low-voltage Teflon-insulated resistance wire. The collector was made of stainless-steel tubing 0.97-cm. (0.38-in.) i.d. and 25.4 cm. (10 in.) long, packed with Teflon powder coated with 10% hydrocarbon grease, mol. wt. about 1000 (Apiezon L). Prior to use, the organic vapors were removed from the collector by heating it in a stream of highly purified helium until a satisfactory base line gas chromatogram was obtained. During the sample collection from the vagina, the collector was kept in an aluminum block at 38° to prevent the condensation of water from vapors. Vaginal vapors were made to flow into the collector by allowing silicone oil to flow from the upper to the lower of the two flasks shown. A dry ice–propanol trap between the collector and the oil prevented atmospheric or oil vapors from reaching the collector. Organic vapors from the vagina dissolved in the hydrocarbon grease coating of the collector, while water vapor passed through.

The other Teflon tube leading to the vaginal insert allowed air to replace that which was removed into the collector. To block room vapors from entering the collector *via* this Teflon tube, room air was first passed through an activated carbon adsorber cooled to −80° by a dry ice–propanol mixture. Usually 2 l. of air was sampled during a collection period of 20 min. In addition to the collected vapors, the Teflon insert with its vaginal debris was used for odor-threshold studies. Immediately after removal from the patient, the insert was placed into an odorless glass vessel provided with inlet and outlet tubing. If odor study on the insert was postponed, the vessel and contained insert were stored in a freezer and brought back to room temperature shortly before study.

Table I—Example of Data Treatment of Vaginal Vapors by a Small Panel [Quantal Response Method (4)][a]

Stimulus Level as Methane Tracer Detector Current, pa.[b]	No. of Panelists Beginning to Detect Odor	Tolerance Level,[c] pa.	Frequency of Observation Rank	Rank $\frac{}{N+1}$
9	0			
30	0	16		
125	4	60	2.5[d]	0.355
360	1	210	5.0	0.72
900	1	570	6.0	0.86

[a] Six panelists, $N = 6$. [b] pa. = picoampere (10^{-12} amp.). [c] Calculated as logarithmic average of the adjoining levels. [d] Ranks 1, 2, 3, and 4; average rank 2.5.

Odor-Threshold Measurements—The statistical design followed a combination of triangle test (3) and quantal response (4) methods. The vapor from the insert with vaginal debris, in known dilutions with nitrogen, was led in a special olfactometer to one of three sniffing ports. In this olfactometer, nonodorous methane carries the vapor into a nitrogen stream. The amount of subsequent dilution by cryogenically deodorized nitrogen is monitored continuously by a hydrogen-flame ionization detector. The detector indicated the methane content by a proportional increase in the ionization current. The measurements permitted calculations of the degree of dilution of odorous vapors in the sniffing port.

The panel of observers sniffed at each of the three sniffing ports by using the statistical design of the ASTM method (5). A negative response was recorded if no odor was reported at any of the ports, or if the observer reported odor at a wrong port. A positive response was recorded if an odor was reported at a correct port (ports were changed randomly). Concentrations of odorous vapors were increased threefold for the next observations, and so on. At each of the concentrations, several observers reported their judgments independently.

Data were tabulated, ranked, and plotted as shown in Table I and Fig. 2. This approach is the same as that used in the study of responses to poisons or other drugs, except that here the response is a positive observation of odor. The concentration level at probability = 0.50 is the odor ED_{50}, the lowest level at which half of the observers would sense an odor. The detector units of the olfactometer were converted to p.p.m. of headspace, using the methane calibration factor.

Gas-Chromatographic Sensory Assay—The vapors in the collector (Fig. 1) were transferred by a special injection needle to a gas chromatograph, using procedures described elsewhere (6). The transfer was effected in a device in which high-purity helium was passed through the collector and injection needle, connected in series with a short length of Teflon tubing. The needle was cooled with liquid nitrogen while a heated copper block traveled along the collector at the velocity of 2.54 cm. (1 in.)/min. in the direction of He flow. The motion was provided by means of a motor-actuated string device (7). Vapors from the collector were eluted in the direction opposite to that which was followed during collection. This method minimized the contamination of the sample by impurities generated by decomposition of the collector's hydrocarbon-grease phase, since each portion of the collector was heated for only a short time.

In the gas chromatograph the carrier gas was He at a flow rate of 10 ml./min. An open tubular column 15.24 m. (50 ft.) long, coated with polyethylene glycol (Carbowax 20 M), was used, and temperature was programmed from 60 to 180° at 4°/min. A hydrogen-flame ionization detector was used, with a sensitivity of approximately 4000

Table II—List of Diagnoses and Samples

Description	Patient Number	Test Number
Normal	1	1
Hematuria	2	2-1 Before Rx[a]
		2-2 4 hr. after Rx
Stress incontinence	3	3-1 Before Rx
		3-2 4 hr. after Rx
Endometritis	4	4-1 8 hr. before Rx
		4-2 Immediately before Rx
		4-3 15 hr. after Rx
		4-4 24 hr. after Rx
Endometritis	5	5-1 7 hr. before Rx
		5-2 Immediately before Rx
		5-3 16 hr. after Rx
		5-4 24 hr. after Rx
Endometritis	6	6-1 7 hr. before Rx
		6-2 Immediately before Rx
		6-3 17 hr. after Rx
		6-4 24 hr. after Rx
Bacterial vaginitis	7	7-1 7 hr. before Rx
		7-2 Immediately before Rx
		7-3 20 hr. after Rx

[a] See *Schedule of Tests* section for definition.

Table III—Odor Thresholds, EG_{50}, of Vaginal Vapors

Case No.	Odor Threshold[a] Vapor in Nitrogen, p.p.m. Before	After	Av. Ratio[b]
2	360	3,400	9.5
3	3,400	80,000	23.0
4	290	480	1.6
5	940		
	15,000	17,000	4.0
		13,000	
6	25,000		
	34,000	42,000	1.1
		25,000	
7	1,700	2,400	2.7
	480		

[a] When two collections of vapors were made, the value for the second collection is placed below that for the first. [b] Odor thresholds averaged geometrically—considered correct procedure for dealing with thresholds covering several orders of magnitude.

pg./cm.[2] of the gas-chromatographic peak area[2] at 0.1 × 1 electrometer setting (the highest sensitivity setting available). A series of *n*-alkanes was run as standard for conversion of retention times to approximate Kovats Indices (8) using a fifth-order polynomial curve and a computer to interpolate values.

At the exit from the gas-chromatographic column 110 ml./min. of He was added to the column effluent and the combined effluent of 120 ml./min. was split 1:1 so that 60 ml./min. was delivered, respectively, to the detector and to a sniffing port. A chemist experienced in this technique observed and recorded the odor of the gas chromatographically separated components (9, 10).

By this technique the components of the vapors are delivered at their respective concentrations at the ports in a sequence which depends on vapor pressures and polarities (in a gas chromatographic sense) of the components. The following concentration relations apply. For example,[3] let the original concentration of a component in the air = *n*-g./ml. At perfect efficiency the collection and transfer from 2000 ml. will deliver 2000 *n*-g. into the gas chromatograph. Of this, 1000 *n*-g. goes to the sniffing port, diluted in He carrier and subsequently, on leaving the port, to some extent also diluted by room air. A typical peak was carried out in 20–40 ml. He, and if the substance was evenly distributed (in mixing with air before reaching the nose), the actual concentration reaching the nose was 10 *n*–20 *n*-g./ml. This was higher, by an order of magnitude than that in the original vaginal air sample. Due to losses in collection and transfer, however, the actual concentration factor was somewhat less.

Potent odorants with low-odor thresholds can exhibit odor at concentrations in the range 0.1–10 pg./ml. of air. Calculations indicate that by collecting organic vapors from 2 l. of air and using a detector with sensitivity on the order of 1000 pg./cm.[2] of chart area, most odor-relevant compounds will be adequately represented in the gas chromatograms. A few, however, still can be odorous without exhibiting visible peaks.

The odor characters of the various components are tabulated in broad categories. The most convenient categorization is a hedonic one; it is based on a scale of "obviously fragrant" and "pleasant" at one end to "objectionable" and "highly objectionable" (repulsive or nauseating) at the other. Between these extremes there is a category of odors of slight, mild, or moderate character. These do not impress observers as being significantly fragrant or objectionable. This intermediate category consists of odors, some of which do not immediately resemble a familiar odor and others which do have an obviously distinct recognizable character ("musty," "sour," "mushroomlike," "burnt," "medicinal," *etc.*).

Because of differences in the character and intensity of odors of the components, the number of distinguishable peaks and their prominence are not the best guides for the comparison of odors of different samples. In addition, some peaks may represent more than one component, each with different sensory characteristics. Most organic materials are odorous at sufficient concentrations. However, some substances, such as hydrocarbons, have high thresholds and can produce large peaks in chromatograms but are not odorous on elution from the sniffing port, and, therefore, contribute little to the initial (mixed) odor. Also, a rich pattern with many larger peaks can indicate strength of an odor but not its objectionability.

Although overall richness can be a guide to the comparison of samples which are similar in composition, much more odor-relevant information is obtained by tabulating peaks by their odor categories and comparing the number of peaks in different categories. As unpleasant odors come under control during treatment, the number of objectionably odorous peaks decreases. Such effects occur even in those chart locations where no pleasantly odorous peaks emerge.[4] In locations where both objectionable and nonobjectionable and/or

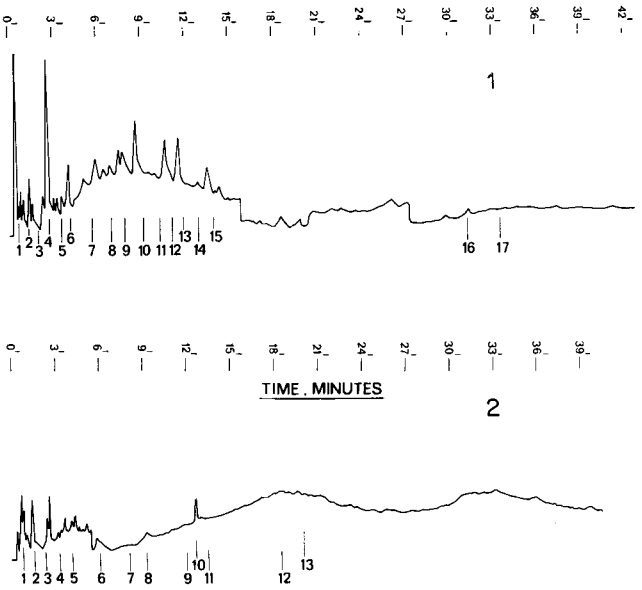

Figure 3—*Typical gas chromatograms of vaginal vapors. 1, Before treatment—odors: 1, pungent unpleasant; 2, very strong, unpleasant; 3, very unpleasant, cut liverlike; 4, very strong; 5, very unpleasant, nauseating; 6, no odor; 7, no odor; 8, very unpleasant; 9, sweet, unpleasant, nauseating; 10, very unpleasant; 11, very bad, nauseating; 12, very unpleasant, nauseating, repulsive; 13, very bad, repulsive, nauseating; 14, very unpleasant; 15, bad; 16, sharp; 17, slight, not unpleasant. 2, After treatment—odors: 1, moderate; 2, very pleasant, caramellike; 3, slight; 4, slight; 5, moderate; 6, slight; 7, no odor; 8, moderate; 9, slight; 10, not unpleasant, 11, no odor; 12, no odor; 13, moderate, not unpleasant. Some "no odor" peaks not listed.*

[2] Picrogram (pg.) = 10^{-12} g.
[3] Where "*n*" is a particular value in the system being analyzed.
[4] *cf.* Table V, Subject 6, Kovats Index range 500–600.

Table IV—Odorograms of Vaginal Vapors (Subjects 1–4)[a]

Kovats Index Ranges	Subject 1	Subject 2		Subject 3		Subject 4			
	1	1	2	1	2	1	2	3	4
<400	V	—		V				0	
400– 500					—	V		0	0
500– 600	X	V							
600– 650					—	VV	X		
650– 700				XL				0,0	0,0
700– 750	X,X	V	—	XL	—	V	X,X		0
750– 800				X		V	X	&,&	0
800– 850			—	X	—	V	X	&	0
850– 900	X,X				&	XXXL	X,X		&
900– 920				X		XXXL			
920– 940			&			XXX	X		&
940– 960	0		&			XXX			
960– 980	0			X		XXX	XXXX	&	0
980–1000	0			X			XXXX	&,&	0,0
1000–1020								&	
1020–1040				X		XXXX	XXXL		0
1040–1060	&	X		V			XXXL	0,0	0
1060–1080	&,&			XXX	&&	XXXX		0,0	0
1080–1100						XXXX		0,0	
1100–1120	XL			XXX		—		0	0,0
1120–1140	0,0	XXX				—	XXXL	&	0,0
1140–1160	X			XXX	&&		XXXL,XXX	&	0,0
1160–1180	X,X		&			—	XXXX	&	
1180–1200	XXX			X		XXX	XXX	&	
1200–1220	XXX,XXX					XXX	XXX	&	
1220–1240					—	XXXX	XXXX	0	
1240–1260	XXXX	XXX							—
1260–1280					0			0	
1280–1300	XXX					XXX	X	—	—
1300–1320	XXX			XXX		XXXX,XXXX	X	—,0	
1320–1340							XXXX	—	
1340–1360	XXXX					XXXX		0	
1360–1380						XXXX			
1380–1400					0	XXXX	X	—	0
1400–1420	0			XXXX		XXX,XXX	—	—	0
1420–1440	XXX							—,0	—
1440–1460				XXXX		XXX			
1460–1480	X							—	
1480–1500					0			—	
1500–1520				W		—			
1520–1540						—,—		0	
1540–1560	X	XXX			0				
1560–1580	XX			X					
1580–1600						—			
1600–1620		XXXX			—	—		—	
1620–1640	0					—	—	—	—
1640–1660		V							
1660–1680	—				—	—,—		(S)	0
1680–1700				XX					
1700–1720	W	XXXX			&	—	X		
1720–1740		X							
1740–1760	0,0					—,—	X	—	
1760–1780	0								
1780–1800						—			
1800–1820	0			V					
1820–1840									
1840–1860									
1860–1880									
1880–1900					&&				
1900–1920									
Seconds:									
1400–1550	XXXb	B		V	&&				
1550–1600				B		—	X		
1600–1650	—			B		—			
1650–1700	—			B	—		X		
1700–1750							X		
1750–1800	—			W		—,V			
1800–1850	0			—	B				
1850–1900	X			W		0			
1900–1950	—			W				B	
1950–2000					B	0	B		
2000–2050	—								
2050–2100	—								
2100–2150					—				
2150–2200									
2200	M			—					

[a] Weak, mild, not unpleasant, 0; unpleasant, X; bloodlike, XL; very unpleasant, XX; strong, sweatlike, XXX; nauseating, repulsively sweet, XXXX; pungent, sharp, V; waxy, W; medicinal, M; earthy, mushroomlike, E; sour, S; burnt, B; peak but no odor, —; in parentheses, odor, but no peak ( ); pleasant, & ; very pleasant, fragrant, & &. Comma separation used if two or more peaks were observed in the range.

Table V—Odorograms of Vaginal Vapors (Subjects 5–6)[a]

Kovats Index Ranges	Subject 5				Subject 6			
	1	2	3	4	1	2	3	4
<400								
400– 500			—			0	—,—	
500– 600	XXX	XXX,V		0	X	XXX	—	—
600– 650	XXX				X,XX		—	—
650– 700			&			X	—	
700– 750	V	XXX,XXX	&	&,&	XXX	0	&	—,—
750– 800	X				XXX	0,0,XXX		—
800– 850		XXXX	0	&		XX		&
850– 900	XX,X		0	&	XXXX	XXXX	&&	&
900– 920				0	—	XX		
920– 940			0					0
940– 960	XX			X		XXXX	0	
960– 980	XXX	M.M	0,M,&			XXX	0	
980–1000	XXX			0,&	XXX	XXXX	&	—
1000–1020					XXX	0	&	
1020–1040	M	M,X,XXXX	—	XXX	XX		&	X
1040–1060	M,M		—	X	XXXX	V	&	0
1060–1080	XXXX	XXXX,XXXX	—	X,X	XXXX	0	—	0
1080–1100	XXXX	XXXX,XXXX,XXX			XXXX	0,0	—,—	0,&
1100–1120	XXXX	XXX,Xb			XXX	0,0	—,—	&
1120–1140		Xb,Xb,Xb	—	X	0,0	E	—	0
1140–1160	X	XXXX,XXXX			0	E,B	—,—,—	
1160–1180	—,—				0	XXX	—,0	
1180–1200		0	0,0,W		0		X,—	—
1200–1220	XXXX	0		0	0			
1220–1240	XXXX			0,0			—	
1240–1260	0	XXXL				S		
1260–1280								X
1280–1300	0,0			—	XXX		—	
1300–1320	—,X	0		0	Xb			
1320–1340								
1340–1360	Xb			0	XXX			
1360–1380		X	0				—	
1380–1400	V			M				
1400–1420					X			
1420–1440			—					
1440–1460							—	
1460–1480	X							
1480–1500		0					—	
1500–1520	XXX		0			XXXX		
1520–1540				0			—	
1540–1560	E						—	
1560–1580			&		XXXX			
1580–1600								
1600–1620	XXXX	&	0	—				
1620–1640					0	XXXX	0	0
1640–1660		V						
1660–1680								
1680–1700		XXXX		—				
1700–1720	0		0		X			
1720–1740						0	—,(0)	—
1740–1760		XXXX			XXX			
1760–1780			0					
1780–1800		B	0		XXXX	X		0
1800–1820	V							
1820–1840								
1840–1860								
1860–1880								
1880–1900								
1900–1920	X				S	XXX	0	—
Seconds:								
1400–1550	V				X	XXXX	0,0	
1550–1600	B	B			—			0
1600–1650	V		B		XXX			
1650–1700	B			0		S		
1700–1750								
1750–1800				—				—
1800–1850	V				B	XXXX	B	—
1850–1900			—	—	V,B		—	0
1900–1950			—					0
1950–2000	B			B				
2000–2050			—		V	B		B
1050–2100	V,—				B	B		B
2100–2150							B	
2150–2200	B			B	0,0	E		
2200	V,B		—,—,—,—	B				

[a] Weak, mild, not unpleasant, 0; unpleasant, X; bloodlike, XL; very unpleasant, XX; strong, sweatlike, XXX; nauseating, repulsively sweet, XXXX; pungent, sharp, V; waxy, W; medicinal, M; earthy, mushroomlike, E; sour, S; burnt, B; peak but no odor, —; in parentheses, odor, but no peak ( ); pleasant, &; very pleasant, fragrant, & &. Comma separation used if two or more peaks were observed in the range.

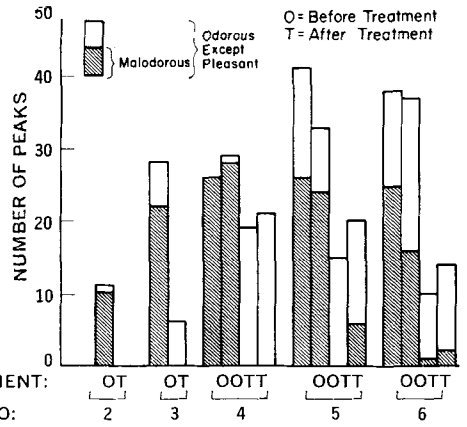

Figure 4—*Changes in number of odorous peaks in vaginal-vapor chromatograms upon nitrofurazone treatment.*

pleasant peaks coexist, and may be only partially resolved, malodor control leads to a decrease of malodorous components. As a result overall odor at that zone of the chromatogram changes from malodorous toward the neutral or fragrant. Also, odorous peaks can become smaller and nondetectable at the sniffing port whenever the odorant there falls below its odor threshold. In essence the process serves as a "spectrograph" for the odorants, with magnification and focussing to aid the odor observations.

Figure 3 illustrates gas chromatograms obtained in a case in which overall odor intensity of vaginal vapors decreased sharply upon treatment with nitrofurazone. The number of components observed in the gas chromatogram also decreased. In this case, the most significant change occurred in the odor character of the peaks:

Chromatogram No.	Number of Peaks			
	Objectionable	No Odor	Neutral	Fragrant
1	14	13	2	0
2	—	4	9	1

Schedule of Tests—Table II shows the diagnoses and the arrangement of test samplings. All subjects were selected from hospitalized patients.

The suppositories (Rx) contained nitrofurazone, 0.3%, in a base of glyceryl monolaurate and polyoxyethylene (4) sorbitan monostearate. They melt at body temperature and are self-emulsifying in vaginal fluids. It is assumed that the 2-g. suppository was spread fairly evenly over the vaginal mucosa. The suppository did not exhibit fragrant or objectionable odor.

RESULTS AND DISCUSSION

Odor Thresholds—Measurements of odor thresholds are summarized in Table III. In all cases the threshold increased upon treatment with nitrofurazone. To make the odor unnoticeable, less nitrogen was needed to dilute the vapors after treatment than before. For the six subjects this difference reaches the 95% confidence level (error probability of 0.05 or less by the Wilcoxon test).

There was a tendency for this effect to diminish as thesholds increased, with a rank-correlation coefficient of +0.74, somewhat short of the +0.83 value required for the 95% confidence level.

Since during odor-threshold studies the insert was at room rather than at body temperature, the absolute threshold values are lower; differences between them, however, are valid for comparisons. Also, the character (quality) of an odor is not considered in measurement of threshold.

Reciprocals of odor thresholds cannot, *per se*, uniquely describe odor intensities. Even in the absence of fragrant components, vapors with lower thresholds do not necessarily have higher odor intensities when not so diluted. The odor threshold data were instructive and indicated that odor was controlled by treatment. However, a better insight can be gained into what occurs with the odor complex during treatment from inspection of the odorograms.

Gas-Chromatographic Assays—Data from odor assays combined with gas-chromatographic separation of vapor components are compiled in Tables IV and V. The Kovats Index coordinates' numerical increase indicates that the vapor pressures of the corresponding odorants were decreasing, or their solubilities in the polar stationary phase (polyethylene glycols)[5] were increasing. No calibration was available toward the end of the scale, where values are listed simply as seconds of retention time and therefore are valid only relatively. Coding of odor characters is indicated in the legends to Tables IV and V.

These odorograms indicate clearly changes in vapor composition associated with use of nitrofurazone suppositories.

Case 1 was that of a normal subject. Vapor-component odors were distributed widely over the range from objectionable (only two peaks were in the most objectionable category) through many neutral to some pleasant components.

In the other five cases investigated, pretreatment odorograms were dominated by components of varying objectionability. After nitrofurazone treatment few, if any, objectionable odorants were observed. Some fragrant notes occurred after treatment, and many components were represented by small peaks unassociated with odor; these components were present at levels far below their odor thresholds. Regions where malodors were present before treatment were not populated by odorous peaks after treatment, or showed peaks with lower odor intensities. These may correspond either to the same substances as were present before treatment, now at lower concentrations, or to new (*e.g.*, fragrant) substances, or to a mixture of the two with objectionable components in part reduced.

As is evident from inspection of Tables IV and V, the effect of treatment cannot be explained by mere masking of unpleasant peaks by fragrant components. In several gas-chromatographic positions where highly objectionable peaks were observed before treatment, no fragrant or other odorous peaks appeared after treatment.

Figure 4 shows the frequency of occurrence of various categories of odors in the vaginal vapors studied, regardless of positions of the gas-chromatographic peaks of these odorants.

The chemical identification of the malodorous components would be significant and should represent a natural extension of this work. However, because of the very low concentrations of the odorants, this is a formidable task and was not undertaken at the present time. For an orientation (see Tables IV and V), the components with Kovats Index of 1000–1100 correspond to the substances boiling in the range of 100–180° and having molecular weight of 80–150. Substances with larger indexes boil higher and have larger molecular weights.

The corresponding relative areas are not useful for comparison of odor relevance since substances vary so greatly in thresholds and intensities of odors. A small peak may be associated with a strong odor and a large peak may be odorless. Even comparison of what appears to be the same peak in two gas chromatograms can be misleading. For example, a peak may correspond to an unresolved mixture of two substances, one contributing most of the area but no odor, the other being responsible for the odor but for only a small fraction of the peak's area. If no odor is observed in the presence of a peak from the sniffing port, this can indicate the presence of components which might still be odorous at a higher concentration.

In odor assays by the technique outlined previously, the method should be considered as a help in separating various odor notes so that they can be surveyed more accurately. The gas chromatograms indicate approximate chromatographic locations of component odorants, convenient for systematization and display.

SUMMARY AND CONCLUSIONS

A technique for gas-chromatographic separation and assay of the components of complex vaginal odors, assumed to be due in large part to bacterial action, was applied to the study of vaginal odors before and after treatment with nitrofurazone antibacterial vaginal suppositories. Odor thresholds changed under treatment in a direction which indicated reductions in the noticeability of odors at a distance. The assays indicated that major changes occurred in the composition of the complex odors, with significant reduction in the occurrences of objectionably odorous components at odor-threshold

[5] *n*-Alkanes are designated by their number of carbon atoms times 100; thus the number for *n*-decane is 1000. Polar compounds' numbers are higher, relative to the number of C atoms (*e.g.*, *n*-butanol's number of 1113).

and suprathreshold concentrations. Under treatment of the vagina with the suppository, the respective odorograms became populated by nonobjectionably odorous components and some fragrant ones which may have been derived from the suppository preparation (although its composition and odor qualities provide no support for this statement). In some gas-chromatographic positions which were populated by malodors before treatment, no odorants were found after treatment; the effect of treatment therefore is not a simple masking but rather the result of simultaneous reduction in malodorous components and increased dominance of milder odors and fragrances.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 27, 1969, from *IIT Research Institute, Chicago, IL 60616*

Accepted for publication December 8, 1969.

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Spectral Studies of Trimethylsilyl Ether of Chloramphenicol

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Abstract □ The trimethylsilyl ether of chloramphenicol used in GLC analysis has been characterized by UV, near IR, IR, NMR, and mass spectroscopy, confirming the structure of the *O,O*-ditrimethyl siloxy derivative. Pyridine and acetonitrile, used as solvents in the silylation reaction, promote formation of diastereomers. Addition of hydroxylic solvents promotes the inversion of configuration and causes partial solvolysis. To verify this, two fractions were collected from a GLC column and analyzed by IR and mass spectroscopy. The experimental evidence presented establishes the two fractions as diastereomers.

Keyphrases □ *O,O*-Ditrimethyl siloxy chloramphenicol diastereomers—structure confirmation, effect of pyridine, acetonitrile □ Trimethylsilyl ether—chloramphenicol derivative, effect of pyridine, acetonitrile □ IR—structure, identification □ UV—structure, identification □ NMR—structure, identification □ Mass spectrometry—structure, identification □ GLC—analysis, separation

Chloramphenicol, D(−)-*threo*-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol, is a certifiable antibiotic for which neither of the current official assay methods (microbiological and UV procedures) (1) is specific. Gas-liquid chromatographic (GLC) procedures were investigated for chloramphenicol assay; however, when chloramphenicol was chromatographed on a QF-1 column above 200°, poorly resolved peaks were obtained, which were probably due to thermal breakdown products (Fig. 1). When it was chromatographed on a DC-200 or SE-30 column, no peaks of any kind were obtained.

Several workers (2–4) have successfully applied the silylation method of Bentley *et al.* (5) to this determination. However, several problems arose when the method was tried in this laboratory. The principal difficulties included contamination of the anode of the flame-ionization detector and solvent tailing, similar to that previously observed in the GLC of lincomycin (6). To overcome these difficulties, the technique was modified as follows: a previously developed extraction procedure (6) was used; methanol or other hydroxylic solvent was added to the sample immediately before injection into the gas chromatograph; different solvent systems were used; and the reaction mixture was evaporated to dryness and reconstituted in an inert solvent.

The TMS derivative prepared by the procedure of Bentley *et al.* (5) exhibited a single symmetrical chromatographic peak at 5.7 min. (Fig. 2) on each of the three columns (7), and recovery was quantitative. Freshly prepared solutions of the derivative gave no other significant peaks when injected directly. After standing (at least overnight), these solutions began to exhibit secondary peaks at 9.2 min., which reached limiting values of 2–3.5% of the area in the case of ethyl acetate or methylene chloride solutions but increased to about 50% in pyridine or acetonitrile. The addition of small amounts of methanol or other hydroxylic solvents tended to accelerate this reaction. With increasing amounts of methanol, additional peaks at 4.3 min. were noted on the QF-1 column (Fig. 3) but not on the DC-200 or SE-30 columns. When the methanol solution of the derivative was partitioned between water and carbon tetrachloride, the chromatogram of the nonpolar fraction showed two peaks (at 5.7 and 9.2 min.; Fig. 4) attributed to the TMS ether of chloramphenicol and its *erythro* isomer, while the chromatogram of the polar fraction was similar to that of the