Evidence for a Chromatographic Model of Olfaction

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ABSTRACT The gradient of activity produced along the olfactory mucosa by odorant stimulation was measured by the ratio (the LB/MB ratio) of the summed neural discharges recorded from two branches of the olfactory nerve, a lateral branch (LB) supplying a mucosal region near the internal naris and a medial branch (MB) supplying a region near the external naris. Twenty-four frogs “sniffed” sixteen different odorants, each odorant at four concentrations and two flow rates. Increases in concentration and flow rate produced statistically reliable increases in the ratios; the magnitude of these increases was considerably smaller than the magnitude of the statistically significant changes that could be achieved by shifting the odorants themselves. Even the small change due to concentration depended upon the odorant presented. Thus, even at the highest physiologically possible concentrations and flow rates, the general level of the activity gradient along the mucosa appeared to be determined mainly by the particular odorant used. The relative retention time of each of these 16 different odorants was measured in a gas chromatograph fitted with a Carbowax 20M column. In general, the longer the odorant’s retention time the smaller its LB/MB ratio. This suggests that the different mucosal gradients of activity are established for different odorants by a chromatographic process. The data further suggest that the mucosa behaves like a polar chromatographic column.

INTRODUCTION
Mozell (1964 a, b; 1966, 1967) has proposed that olfactory discrimination, in analogy to chromatography, depends in part upon the facility with which the molecules of each odorant can migrate along the mucosa. Other authors have at times suggested similar mechanisms. Adrian (1950, 1951), in order to explain his observation of electrophysiological spatiotemporal differentiations of odorants in the olfactory bulb, proposed that the molecules of different odorants might spread in different spatial and temporal patterns along the mucosa in accordance with their diffusion rates and mucus solubilities. However, he later deemphasized this mechanism in favor of a selective sensitivity of the receptors themselves (Adrian, 1953, 1954). Nevertheless, Beidler...
(1957) continued to point out that the molecules of some chemicals may be bound less strongly to the olfactory receptors than are the molecules of other chemicals and will consequently travel farther and faster along the mucosa. Moncrieff (1955) had earlier presented evidence for such a differential adsorption of different vapors along the nasal epithelium (though not specifically along the olfactory epithelium). He observed that different odors passed through the nasal passageways of freshly sacrificed sheep at different rates. However, he further showed that the differential adsorption of odors likewise occurs on nonolfactory tissues as well as on nonbiological materials. Indeed, whenever molecules of different chemicals pass over any surface, they are likely to do so at different average rates. Thus, to maintain that differential molecular migration has significance in olfactory discrimination, it was necessary to demonstrate not only the differential ability of the molecules of different chemicals to migrate along the mucosa but also to show that this difference is reflected in the neural discharges transmitted centrally.

Thus, Mozell (1964a, 1966) sampled the activity occurring in two separate regions of the frog olfactory mucosa in response to four different odorants by recording from the branches of the olfactory nerve which supply these two regions. One region was nearer the external naris and was therefore contacted earlier by the incoming odorized air. The other region, nearer the internal naris and therefore further along the flow path, was contacted later. In order to compare the relative magnitudes of the activity elicited in each of the mucosal regions in response to four different odorants, Mozell measured (a) the ratio of the multifiber discharge magnitudes recorded from the two nerve branches and (b) the difference between the discharge latencies of the two nerve branches. Since the magnitude of these measures was found to depend upon the chemical presented, Mozell suggested that these differential patterns, which reflect the various gradients of activity along the mucosa, might operate as one of the basic mechanisms in olfactory discrimination.

How might different chemicals produce these different patterns? First, there may be a disproportionate variation from one region of the mucosa to another in the relative numbers of receptors selectively sensitive to each of the chemicals. Second, the molecules of some chemicals, having a greater facility to migrate along the mucosa, could reach certain regions more rapidly and in greater numbers than the molecules of other chemicals. Mozell (1964a) favored the latter explanation since it was more consistent with his observation that a reversal in the direction of odorant flow along the mucosa led to a reversal in the relative magnitudes of the responses recorded from the two nerve branches. Thus, the process of analysis and separation of chemicals by olfaction was likened to other processes which, in order to analyze and separate chemicals, take advantage of the differential facility with which different classes of molecules can migrate, viz., chromatography.
As further support for this analogy, Mozell (1966) observed that the latency differences and the LB/MB ratios produced by these four chemicals seemed to parallel, to some degree, their retention times as measured by other investigators using commercially available gas chromatographs. In other words, these preliminary observations suggested that the longer the transit time of molecules through the chromatographic column, the later and smaller the response at the farther mucosal region.

Thus far Mozell has based his argument for a chromatographic model for olfactory vapor analysis upon quantitative studies involving only four chemicals. Obviously, this model must be tested with a larger number of chemicals. Thus, it was the purpose of this study to determine whether the chromatographic model of olfaction can be generalized beyond the four odorants which led to its proposal.

METHODS

Recording A more detailed description of the recording techniques can be found in an earlier paper (Mozell, 1966). Louisiana bullfrogs (*Rana catesbeiana*) were anesthetized with urethan; stainless steel electrodes (enameled wires 63.5 μ in diameter) and Grass P5 AC preamplifiers were used to record the neural activity from the most lateral (LB) and most medial (MB) olfactory nerve branches coursing across the dorsal aspect of the frog's olfactory sac. MB supplies the mucosal region near the external naris and LB supplies a region near the internal naris (Mozell, 1964 a). Care was taken to keep the olfactory sac intact, thus ensuring the integrity of the normal air flow path along the olfactory receptor sheet.

Quantification of the activity recorded from each nerve branch in response to stimulation was accomplished by leading the preamplifier outputs through summator (integrator) circuits, the outputs of which were in turn used to drive the galvanometers of a Honeywell Visicorder. As discussed by Beidler (1953), the areas under the resulting traces can be taken as proportional to the neural discharge magnitudes and accordingly they were measured with a planimeter. The area under the trace produced by the lateral branch divided by that produced by the medial branch yielded a ratio (the LB/MB ratio) which indicated the steepness of the gradient of activity across the mucosa for each odorant. The smaller the ratio the sharper the decrease in activity from the region near the external naris to the region near the internal naris.

Stimulus Control A schematic diagram and detailed description of the olfactometer and the technique used to produce controlled artificial sniffs have been published previously (Mozell, 1966). Briefly, the required odorant concentrations (expressed in terms of partial pressures) were produced with a flow dilution olfactometer in which an air stream was first saturated with an odorant at a given temperature and then diluted as needed with an independent, nonodorized air stream. Samples of this final mixture with controlled odorant concentrations were drawn through a frog's olfactory sac as artificially produced sniffs of known volume and flow rate. This was accomplished by using a Harvard withdrawal pump to draw a slug of the mixture into one of the frog's nares via a side arm sampling the main air stream of the olfac-
The volumes ranged from 0.2 to 0.6 cc for different animals but were held constant for any given animal. Half the animals were tested with flow rates of 20.6 cc/min and the other half with 4.12 cc/min.

Since some parts of the olfactometer and its link to the frog necessarily became contaminated with the odorant being presented (see Fig. 1, Mozell, 1966), these parts were routinely disconnected and replaced after each stimulation.

**Stimulus Presentation** Sixteen different chemicals were used as odorants, each presented at four different partial pressures and two different flow rates. The chemicals were: n-octane, benzaldehyde, n-butyl acetate, d-limonene, furfurol (furfuryl alcohol), n-butanol, n-nonane, carvone, 4-heptanone, amyl acetate, heptaldehyde, geraniol, isovaleric acid, diphenyl oxide, citral, and methyl benzoate. All the odorants were presented at the same two flow rates (4.12 cc/min and 20.6 cc/min). Since, however, the vapor pressures of several of the odorants differed considerably from those of the majority at the ambient temperature (23°C ± 1°C), they could not all be presented at the same partial pressures and still have equivalently wide ranges covered. Thus only the first 11 odorants cited above were presented at 0.56, 2.5, 12.0, and 56.0 × 10⁻² mm Hg. Methyl benzoate and isovaleric acid were presented at the same three lower partial pressures but the highest partial pressure available for the former was 35.0 × 10⁻² mm Hg and for the latter 12.0 × 10⁻² mm Hg. For geraniol, citral, and diphenyl oxide the whole range had to be lowered; each was presented at 0.25, 0.56, 1.2, and 2.5 × 10⁻² mm Hg.

In preparing the experimental paradigm for the presentation of the odorants to the animals two special problems had to be considered. First, the total number of presentations to any one animal had to be curtailed because of the total number of stimulations needed and a necessary interstimulus interval of 2.5 min. This interstimulus interval was used to disassemble the apparatus after each stimulation and to prepare for the next. Second, it was necessary to minimize the total number of fresh olfactometer parts needed to replace those which had become contaminated during stimulation.

Twenty-four animals were divided equally into two groups; one group was given nine chemicals (octane through carvone as listed above plus methyl benzoate) and the other was given seven (4-heptanone through citral). Half the animals in each of these groups were given the stimuli at a flow rate of 4.12 cc/min and the other half at 20.6 cc/min. Therefore, there were four groups, each composed of six frogs and each representing a different combination of flow rate and chemical.

In order to conserve olfactometer parts the first odorant was presented at its lowest partial pressure, followed by the second odorant also at its lowest partial pressure, etc. After all the chemicals had been presented at their lowest concentration, each was then presented at its next higher concentration until all the concentrations had been used. The order in which these chemicals were presented was varied from animal to animal. However, the following lists the commercial sources and catalogue numbers. From Eastman Organic Chemicals (Rochester, N.Y.): n-octane, P1107; benzaldehyde, 30; n-butyl acetate, 710; d-limonene, 1980; furfuryl alcohol, 1360; 4-heptanone, P1303; heptaldehyde, 284; geraniol, T378; isovaleric acid, 459; citral, P932 and methyl benzoate, 317. From Fisher Scientific Co. (Pittsburgh, Pa.): phenyl ether, D-89 and n-butanol, A-399. From K & K Laboratories Inc. (Plainview, N.Y.): carvone, 14810. From Humphrey Chemical Co. (North Haven, Conn.): n-nonane. From J. T. Baker Chemical Co. (Phillipsburg, N.J.): amyl acetate, 9094.
animal. When each of the seven or nine chemicals had been presented to a frog at all four partial pressures, the entire procedure, with fresh olfactometer parts, was repeated so that each frog received each stimulus twice. A stimulus was presented a third time only if the ratios yielded by its two previous presentations differed by an arbitrary factor > 0.20.

As the first stimulus in each series of chemicals at a given partial pressure, the frog was given an artificial sniff from the nonodorized air stream alone to check for an otherwise unsuspected contamination of the apparatus and for possible residual odors in the frog’s naris. If there was a response to this air stimulus, the olfactometer was examined for contamination and nonodorized air was drawn through the naris for 1 min. These procedures were repeated until the response to air could no longer be observed.

In order to control for variations occurring over time in the physiology or the recording conditions of the preparation, which might affect the two nerve branches differentially, a standard stimulus was presented to the animal at regular intervals during the course of each experimental session. The standard chosen was d-limonene at a partial pressure of 2.5 × 10⁻² mm Hg and a flow rate of 8.24 cc/min. When this standard was presented for the first time at the beginning of each experimental session, the preamplifier gains were set so that the traces recorded from the two nerve branches were equal; i.e., the LB/MB ratio was set at unity. As the experiment continued, a variation from unity at these gains in response to this standard indicated a change in the preparation which affected the recordings from the two nerve branches disproportionately. The ratio produced by every other odorant was then mathematically corrected for this disproportionate change over time by comparing it to the ratio produced by that standard given at about the same time in the experimental session. That is, each of the LB/MB ratios was divided by the average of the standard d-limonene ratios just preceding it and just following it. In this way the LB/MB ratio produced by each stimulation was corrected by an amount proportional to the relative change in the responses of the two nerve branches. In addition, making all the ratios proportional to that of a standard stimulus essentially adjusted the ratios in different animals to the same scale. This allowed the direct comparison of ratio sizes across animals.

The data from the first 24 animals lasting through the entire experiment have been included in the analysis of the results regardless of the trends they showed. In no case was only part of the data from a given animal included.

**Gas Chromatographic Determinations** The uncorrected retention times of all 16 chemicals were determined by injecting 0.5 μl samples of each into a Varian-Aerograph model 600D chromatograph (Varian Aerograph, Walnut Creek, Calif.) equipped with a flame ionization detector. This was done with each of two columns, one polar (20% Carbowax 20M stationary phase on a Chromosorb P support) and the other nonpolar (5% SE-30 stationary phase on a Chromosorb W support). Four chromatograms using the Carbowax column are given in Fig. 1 as examples. The uncorrected retention time of methyl benzoate was assigned the value of 1.0 and the uncorrected retention times of the remaining chemicals were expressed relative to this standard.

The recorder of the gas chromatograph was set to read 10 mv for a full-scale de-
flection of 10 cm. Then the gain of the detector amplifier was set, for each chemical, to the highest amplification which did not drive the trace deflection off scale. Four of the sixteen chemicals (geraniol [Fig. 1], citral, anyl acetate, and d-limonene) were found to yield more than one chromatographic peak, defined as any deflection greater than 0.1 cm on the recording scale. Consequently, in the graphs comparing the relative retention times of the chemicals to their electrophysiologically determined LB/MB ratios, each of these four chemicals is represented by two retention times; viz., its shortest and longest.
RESULTS

Several examples of the visicorder traces representing the summated neural discharges which are used to calculate the LB/MB ratios are shown in Fig. 2. Other examples of such traces can be found in earlier publications (Mozell, 1964a, b, 1966, 1967).

![Graph showing examples of visicorder traces](image)

**Figure 2.** Examples of the Visicorder traces representing the summated neural discharges which were used to calculate the LB/MB ratios. All these responses came from the same animal. The upper response in each pair was recorded from the lateral branch of the olfactory nerve and the lower response was recorded from the medial nerve branch. The partial pressures are given along the top in terms of $10^{-2}$ mm Hg. In this animal the stimulus volume was 0.4 cc. The flow rate was 4.12 cc/min. The duration of stimulation was 5.8 sec. The onset of the stimulus is shown by the first rise in the stimulus trace and the end of stimulation is shown by the first fall of this trace. Subsequent short-term changes in this trace are artifacts of the stimulus marking system and do not mean further stimulation. The time scale represents 10.0 sec.
The over-all median LB/MB ratios for all animals produced by the different chemicals at each of the eight combinations of two flow rates and four partial pressures are summarized in Fig. 3. Table I presents the results of an analysis of variance statistically testing the significance of each of these primary variables and each of their interactions in the determination of these
LB/MB ratios. Any one of the eight graphs in Fig. 3 makes clear that when different chemicals are equated in concentration and flow rate, they can still produce widely differing gradients of activity along the mucosa as measured by their LB/MB ratios. As shown in Table I, the importance of different chemicals in this regard is corroborated by the highly significant variance ratios \((F)\) calculated between them. The probability \((P)\) of obtaining this difference by chance alone is <0.005.

**Table I**

| Source of variation               | Sum of squares | df | Mean square | \(F\)  | \(P\)   |
|----------------------------------|----------------|----|-------------|------|--------|
| Between groups                   | 95.68          | 95 | 1.007       | 21.94| <0.005 |
| Within groups                    | 21.91          | 477| 0.0459      |      |        |
| Total                            | 117.59         | 572|             |      |        |
| Between chemicals                | 87.38          | 11 | 7.944       | 173.07| <0.005 |
| Between partial pressures        | 2.50           | 3  | 0.8333      | 18.15| <0.005 |
| Between flow rates               | 0.78           | 1  | 0.78        | 16.99| <0.005 |
| Interaction: chemical \(\times\) partial pressure | 3.02 | 33 | 0.0915 | 1.99  | <0.005 |
| Interaction: flow rate \(\times\) partial pressure | 0.14 | 3  | 0.0467 | 1.02  |        |
| Interaction: chemical \(\times\) flow rate | 0.88 | 11 | 0.0800 | 1.74  |        |
| Interaction: flow rate \(\times\) chemical \(\times\) partial pressure | 0.98 | 33 | 0.0297 | 0.647 |        |

*This analysis includes the data from all chemicals except geraniol, citral, diphenyl oxide, and isoalveic acid which were eliminated because they were not presented at the same vapor pressures as the other twelve. A smaller analysis was carried out for those chemicals and its results do not differ significantly from the larger one given above.

† Since 3 of the possible 576 median ratios could not be determined, these were estimated from the other data and the degrees of freedom were adjusted accordingly.

The chemicals in these eight graphs could have been plotted in any order along the abscissa without detracting from the result that different chemicals can yield different ratios. However, when plotted as shown, in the order of the log of their retention times on a Carbowax column, they tend to fall into the order of decreasing LB/MB ratios. In other words, the ratio that a chemical produces appears inversely related to its relative retention time on a Carbowax column. The curves fitted to these data by the method of least squares are intended to show a general inverse relationship only and are not intended to imply necessarily a straight line function. (Indeed, higher degree polynomial curves may be fit to the data of several of the graphs, but it seems premature to try to establish the exact shape of the curve.) Thus, the longer
it takes a chemical vapor to travel through a gas chromatograph fitted with a Carbowax column the greater is the decline in activity from the mucosal region near the external naris to that of the internal naris. This general relation was corroborated by the Pearson product moment correlation coefficients given in the legend of Fig. 3. The high statistical significance of these correlations is shown by their $P$ values which are all $<0.005$. That these 16 different chemicals did not show even greater negative correlations between ratio and retention time was due mainly to one chemical, butanol, which consistently produced a very small ratio in spite of its comparatively short relative retention time.

A comparison of the ratios produced by the different chemicals as partial pressure is increased indicates that the ratio is dependent not only upon the chemical itself (or more correctly its retention time) but also upon its concentration. Although chemicals which give a high, low, or some intermediate ratio value at one partial pressure tend to do so at other partial pressures, an obvious drift towards higher ratios does occur with increasing partial pressures. As shown in Table I this effect of partial pressure is statistically highly significant. Thus, the chemical seems to set the general size of the ratio but its concentration can modify that ratio to some extent. However, the degree to which the ratio can be modified by concentration is greater for some chemicals than for others. For example, at the 20.6 cc/min flow rate the octane ratio shows no consistent change with concentration whereas methyl benzoate shows a very slight over-all increase, and butyl acetate shows a rather marked ratio increase (Fig. 3). Thus, in addition to the effect of each of the variables alone (i.e., chemical and concentration) the interaction between these variables also appears to affect the ratios (Table I; chemical $\times$ partial pressure interaction).

As with partial pressure, Fig. 3 and Table I show that flow rate affects the ratio. A comparison of the two flow rates at any partial pressure shows that although the general size of the ratio is again set by the particular chemical, it may be increased by increasing the flow rate. Although this effect of flow rate alone is statistically significant, its interaction with chemical (unlike the interaction of partial pressure and chemical) does not quite reach significance. Therefore, although the ratio does indeed vary with both flow rate and chemical when each is considered separately, any differences from chemical to chemical in the way flow rate appears to alter the ratios in Fig. 3 are inconsistent enough to be considered statistically a matter of chance.

The interaction of flow rate and partial pressure is not significant (Table I). Thus, there appears to be no statistically significant tendency for increases in partial pressure to produce greater ratio increases at the lower flow rate than at the higher flow rate even though the graphical data (Fig. 3) may in some cases suggest such a tendency.
The triple interaction, which tests whether the interaction between partial pressure and flow rate has a greater effect with some chemicals than others, was not statistically significant.

Although each of the three major variables (chemical, partial pressure, and flow rate) had a significant effect individually, the interaction between these variables was not significant. For instance, to derive the data for (A), all the ratios produced by all the chemicals at both flow rates were averaged for each of the four partial pressures shown. Thus, the effect of partial pressure per se upon the LB/MB ratio could be plotted. The same process, appropriately applied, was used to plot the LB/MB ratio as a function of flow rate (B) and as a function of chemical (C). For meaning of symbols in (C) see top of Fig. 3. The fitted curve in (C) does not include butanol. The correlation coefficient without butanol is \(-0.87\); with butanol it is \(-0.74\). \(P < 0.005\) for both correlations.

**Figure 4.** The average LB/MB ratio as a function of each of the three major variables taken across the remaining two variables. For instance, to derive the data for (A), all the ratios produced by all the chemicals at both flow rates were averaged for each of the four partial pressures shown. Thus, the effect of partial pressure per se upon the LB/MB ratio could be plotted. The same process, appropriately applied, was used to plot the LB/MB ratio as a function of flow rate (B) and as a function of chemical (C). For meaning of symbols in (C) see top of Fig. 3. The fitted curve in (C) does not include butanol. The correlation coefficient without butanol is \(-0.87\); with butanol it is \(-0.74\). \(P < 0.005\) for both correlations.
and flow rate) appears to have a real effect upon the ratio because they all achieved statistical significance, the magnitude of each of these effects requires further analysis.²

Fig. 4 brings this analysis into sharper focus than does Fig. 3 by averaging the ratios produced by each of the variables taken over the other two variables for all animals. For example, Fig. 4 A illustrates the increase in the ratio, averaged across all animals and all flow rates, as the partial pressure increases. The slopes of the curves in Fig. 4 emphasize that there is an overall change in ratio with variations in both concentration (Fig. 4 A) and flow rate (Fig. 4 B) but that, at least over the ranges used in this experiment, these changes are small compared to those engendered by substituting the chemicals themselves, a process which, of course, substitutes the retention times along the abscissa (Fig. 4 C).

DISCUSSION

As stated above, the previous arguments leading up to the proposal of chromatography as a model for olfactory discrimination have been based upon demonstrations involving only four chemicals. This small number of odorants by itself justified caution in evaluating the proposal. Although the sample in this present experiment includes only a small number of all the possible odorants that could have been presented, it is large enough to substantially reduce the probability that the chemicals might fall into some particular relationship by chance alone. Therefore, this larger sample confirms that different odorants can elicit different characteristic patterns of relative response magnitudes from two different branches of the olfactory nerve which supply widely separated regions of the olfactory mucosa. This is not to say that each odorant produces a unique pattern, for as can be seen from the figures, two or more odorants may develop similar patterns. Whether these similarities reflect the actual biological situation, or whether they occur because the techniques are not precise enough to reliably separate chemicals that yield similar but not identical patterns, is yet to be determined.

This chromatographic model does not deny other possible mechanisms for olfactory discrimination. The individual receptor itself has been shown to have different sensitivities to individual odorants, and these spectra of odorant sensitivities also differ between receptors (O'Connell and Mozell, 1969; Gesteland et al., 1965). These two possible discrimination mechanisms, analysis across regions and analysis by individual receptors, need not be mutually exclusive, and by operating together could even generate a combined code with considerably more permutations. Such a code can more adequately represent the vast number of odorants that can be discriminated.

² It will be recalled that statistical significance states whether an effect is more probably real than due to chance, but does not necessarily state the size of the effect.
What is the mechanism for this apparent ability of different chemicals to establish different patterns of activity along the mucosa? The results of the experiment already noted in which the direction of odorant flow was reversed (Mozell, 1964 a), seemed to favor a process involving the differential abilities of the molecules of different chemicals to migrate along the mucosa rather than to favor the possibility of differing geographic distributions of receptors with similar selective sensitivities. However, there are other possible explanations for these gradients of activity. For instance, perhaps the initial receptors, once activated, inhibit the responses of the receptors farther along the flow path, the magnitude of this forward inhibitory effect varying according to the chemical presented. Although tight junctions have been observed between neighboring supporting cells and between supporting cells and receptor cells (Reese, 1965), there is not sufficient electrophysiological evidence to permit this anatomical data to arouse more than a suspicion that currents might flow from olfactory receptor to olfactory receptor via the supporting cells.

On the other hand, the concept that the mucosal patterns are based upon the facility with which the molecules of different chemicals can migrate along the mucosa not only explains the reversal effect but also does so by employing a well-documented physical phenomenon. That is, the molecules of one substance, when allowed to do so, will migrate along a liquid or solid surface more rapidly and reach a given point in greater numbers per unit time than will the molecules of another substance. This phenomenon, under the name of chromatography, has become an extremely useful laboratory technique to separate and identify different chemicals. Perhaps the olfactory system has evolved to take advantage of this same chromatographic effect for the same purpose of identifying different chemicals. In addition, the data presented here give added credence to the suggestion that chromatography may be basic to the generation of mucosal patterns by demonstrating a significant correlation between the different patterns produced by 16 different chemicals and the manner in which these same chemicals are separated by an established chromatographic method.

Some difference in the ability of the mucosa and a Carbowax column to separate odorants was, of course, to be expected. Even then, the correlations observed seem quite remarkable, especially when the effect of butanol, which due to its very anomalous behavior tended to lower the correlation considerably, is taken into account. Although the medial nerve branch almost always responded to butanol, the lateral nerve rarely did. The result was a small ratio in spite of a comparatively short Carbowax retention time. One cannot help but note in this regard butanol's comparatively high solubility in water. With such a high solubility in water and thus, presumably, with such a high mucus solubility almost all the molecules of butanol might be removed from
the odorized air sample upon initial contact with the mucosa, and few, if any, might reach the more distant region served by the lateral olfactory nerve branch. Other highly water-soluble chemicals producing small LB/MB ratios, such as furfurol, which might have been used to further test this point, unfortunately also had long retention times so that they did not, unlike butanol, differentiate the effects of water solubility and retention time. Therefore, at this time butanol's anomalous behavior must remain unresolved. However, it does emphasize that, even granting an analogy between the mucosa and chromatography, it may not be possible to fully characterize the nasal "column" by reference to any specific commercially available one. A more accurate analogue may be a hybrid column combining some of the properties of both Carbowax and mucus. Indeed, any nasal column might include a mixture of several stationary phases such as the mucus, the receptor cells and their cilia, and the supporting cells. Although the exact characterization of the presumed olfactory chromatographic column is not possible at present, it does appear to behave more like a polar column than a nonpolar column.

As shown in Table II, the correlation between the LB/MB ratios and the retention times on a polar column, Carbowax 20M, is much greater than that between the ratios and the retention times on a nonpolar column, SE-30.

The proposed analogy between chromatography and olfactory discrimination at the mucosal level is made only in regard to the basic principle of differential molecular migration; the details of the application of this principle in olfaction and in the various chromatographic techniques may be quite different. For example, in gas chromatography a sample of chemical vapor is injected into a continuously unidirectional flow of carrier gas. The molecules of the sample are then carried along the medium of the column toward a single detector at the end. The time required for these molecules to reach this one detector (the retention time) characterizes a given chemical in gas

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**Table II**

| Retention time on Carbowax 20M | Retention time on SE-30 |
|-------------------------------|------------------------|
| Including butanol             | $r = -0.74$            | $r = -0.21$ |
|                               | $P < 0.01$             | $P > 0.05$  |
| Excluding butanol             | $r = -0.89$            | $r = -0.30$ |
|                               | $P < 0.01$             | $P > 0.05$  |

$r$, product moment correlation coefficient; $P$, level of significance of $r$.

* For explanation of average LB/MB ratio see Fig. 3 legend.

† The Carbowax 20M column supported by Chromosorb P is a polar column.

‡ The SE-30 column supported by Chromosorb W is a nonpolar column.
chromatography. These retention times depend upon a relation common to all chromatography, i.e., the amount of time the sample molecules spend in association with the medium relative to that which they spend in association with the carrier. Although it is being proposed that this relation applies to the olfactory mucosa, in its natural operation there is no unidirectional constant flow and there is not just one receptor placed at the end of the column. Instead, the flow is bidirectional and pulsatile, and a sheet of detectors, the receptors, is spread along the entire column. Because of this pulsatile bidirectional flow, any "nasal chromatograph" would probably not be able to use efficiently a measure based upon the time required to reach the end of the column, since some of the chemicals may not be able to reach that point before the direction of flow is reversed. In a nasal chromatograph, however, the receptor sheet constituting the column could simultaneously signal the distance the molecules have spread along the mucosa and the relative number of molecules reaching each point as they did so. This would be encoded by the patterns of activity elicited along the mucosa. Such a readout better resembles thin layer chromatography than gas chromatography. Obviously, there is no necessity for the nasal mucosa to be similar in its operational details to any one type of commercially available chromatograph.

Whenever a set of neural discharges proposed as a quality code is also found to depend upon stimulus intensity, it is necessary to consider whether this proposed code is so confounded with intensity effects as to be useless for quality discrimination. Thus, there was concern when previous work (Mozell, 1966) gave some indication that the LB/MB ratios increase with increasing concentration, but the data were too fragmentary to establish the reliability of this effect, its extent, and whether it was large enough to greatly interfere with the encoding of quality. However, the statistical and graphical analysis of the present study has clearly demonstrated this concentration effect and, in addition, has uncovered a similar effect due to flow rate increases. On the other hand, this present study has also shown that on the average these effects, over the ranges used, are not large enough to force the abandonment of the gradients of activity across the mucosa as a possible mechanism for odorant encoding.

Of course, it could be argued that if the concentrations were greater than those used in the present study, the patterns produced by different chemicals might then become indistinguishable. However, such an argument must take into consideration that, due to the relation between vaporization and temperature, there is an upper limit to the concentrations that need be suspected of confounding these gradients. In nature this upper limit is imposed by the temperature of the animal's surroundings or occasionally by the temperature of his nasal passageways. In order to test whether higher concentrations could obliterate the patterns, therefore, each chemical was presented to two addi-
tional frogs at the highest partial pressure it can attain at the room temperature (23°C ± 1°C). With so few animals it is difficult to compare the absolute magnitude of the median ratio produced by each chemical in these two additional experiments to those produced by the same chemical in Fig. 3. However, Fig. 5 clearly shows that the LB/MB ratios even at these very high concentrations still depend upon the retention times of the individual chemicals.

There is also an upper limit to the flow rates that must be considered. At flow rates appreciably above 20.6 cc/min the olfactory sac, which in this experiment is not supported on its dorsal wall, can be seen to move during the artificially produced sniff, thus introducing artifacts into the neural recordings. Whether or not similar movements occur in the intact animal is not known but at least they suggest that flow rates much above 20.6 cc/min place a mechanical strain upon the system and, perhaps, should be avoided. In addition, attempts to measure with small rotameters the approximate normal inspiratory flow rate of a frog have shown it to be at about the same magnitude. As shown in Figs. 3 and 4, up to 20.6 cc/min at least, the flow rate effect, although consistent, is quite small.

Although on the average the magnitude of the concentration effect is
small, the degree to which the ratios do change with concentration varies from chemical to chemical. This leads one to speculate that those chemicals which display this concentration effect would also, if tested appropriately, appear to change in odor quality as their concentrations are altered. Such variations in odor quality with changes in concentrations, although part of olfactory "folklore," have not been systematically or extensively studied. The present data tend to confirm the existence of such an effect and would in addition provide a basis for predicting the chemicals for which this effect would be most pronounced.

In attempting to explain the increase in ratio with increases in concentration and flow rate, one could recognize that increases in flow rate and increases in concentration share a common effect, viz., they both increase the number of odorant molecules per unit time to the olfactory mucosa. For some chemicals, then, an increase of either flow rate or concentration may begin to overload the mucosal column (especially at its initial region) thus shunting more and more molecules farther along. This, in turn, would lead to larger ratios. However, still troublesome is the nonsignificance of the interaction between flow rate and chemical which, if this effect of overloading were the sole explanation of the ratio increase, would be expected to parallel the highly significant interaction between partial pressure and chemical.

Finally, it should be pointed out that although sensory coding is generally thought to be based upon a selective tuning of the receptors themselves, a spatial analysis of olfactory stimuli, as proposed here, is not without parallel among other sensory systems. In audition, for instance, the analysis of frequency is made spatially along the basilar membrane in accordance with physical principles not at all peculiar to the receptors themselves. This paper suggests an analogous situation in olfaction.

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