THE OLFACTORY STIMULATING EFFECTIVENESS OF HOMOLOGOUS SERIES OF SUBSTANCES STUDIED IN THE FROG

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The relation between homologous odorous substances and olfactory stimulating effectiveness has been studied by several workers. Chadwick and Dethier (1947, 1949) studied the stimulating effectiveness of a series of aliphatic alcohols, aldehydes and ketones on the tarsal chemoreceptor of the blowfly, and Dethier (1951) tried to analyze the above result from the thermodynamic point of view. Dethier and Yost (1952) studied the same subject on the olfaction of the blowfly. Ottoson (1958) studied the relationship between the activity of the olfactory epithelium of the frog and the physico-chemical properties of odorous substances.

The authors have studied the activity of the olfactory epithelium and bulb in the frog (Takagi and Shibuya, 1959, 1960 a, b, c, 1961: Takagi, Shibuya, Higashino and Arai, 1960), and found that the amplitudes and the shapes of the potentials in these tissues are different to different odours and that the stimulative effectiveness of two odours can be compared by successive application.

In this paper, the stimulating effectiveness of homologous alcohols, acetates and ethers are comparatively studied on these potentials. The relationship between the stimulative effectiveness and the physico-chemical properties of the odorous compounds is considered.

METHOD

The slow potentials of the olfactory epithelium were led by means of glass electrodes with tip diameter of about 100 µ and filled with Ringer's solution. The induced waves of the olfactory bulb were led by means of silver wire electrodes. These potentials were recorded with an ink-writing recorder through a D.C. amplifier and R.C. amplifiers respectively.

Normals and isomers of alcohols, acetates and ethers were used as stimulants. The saturated or diluted vapours were blown onto the olfactory epithelium of the frog (Rana nigromaculata). In order to make the vapours of various concentrations, pure air was added to the saturated vapours or distilled water was added to the original solutions. The duration of odour stimulation was 1 second in most cases. Since the amplitude of the slow potential increases in accordance with an increase of odour

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intensity, the amplitude was taken as an index of olfactory stimulating effectiveness. The whole experiments were performed in the room temperature of 17° to 19°C. The purest compounds commercially available were used.

RESULTS

1. Comparison of the stimulating effectiveness of homologous substances by successive application method. In order to compare olfactory stimulating effectiveness, two kinds of saturated alcohol vapours were applied successively with a pause of 5 sec. It is known that the after-effect of olfactory stimulation remains for more than ten seconds when a strong odour or an odour of high concentration is applied (OTTOSON, 1956). This means that in our experiment the second stimulation was applied in the relatively refractory period following the first stimulation. Consequently, if the stimulative effectiveness of two alcohols are the same, the second slow potential becomes smaller than the first one. In other works, if the second slow potential is bigger than the first one or at least as big as the first one, the second alcohol has much stronger stimulatory effect than the first one. Thus, the stimulative effectiveness of C₃-alcohol is found to be larger than that of C₂-alcohol (FIG. 1). After this experiment, the order of application of the two alcohols was reversed in order to confirm the above results (TAKAGI & SHIBUYA, 1961). For instance, C₃-alcohol was applied after C₂-alcohol, and then after an enough pause the latter was applied after the former (FIG. 1). The second slow

![Fig. 1. Comparison of the amplitudes of the slow potentials and the induced waves produced by various alcohols.](image-url)

The saturated vapours of two homologous alcohols were successively applied for one second respectively with an interval of five seconds. In each group, the top record shows the slow potential of the olfactory epithelium and the bottom one the induced wave of the olfactory bulb. Short horizontal bars indicate the times and durations of application. Explanation in the text.
potential due to C_2 was again smaller than the one due to C_3, and the above result was reaffirmed. By repetition of this method, the stimulating effectiveness were compared among homologous alcohols. A similar result was also observed in the induced wave, as well (Fig. 1). These results are entirely coincident with the results obtained by single application method as shown in Fig. 3. The same experiments were performed in case of acetates and ethers. The results are summarized as follows:

In alcohol series,
\[ C_2 \leq C_1 \leq C_8 < C_7 < C_4 < iC_3 < C_9 < iC_5 < iC_3 < C_5 < C_6 \]

When the stimulating effects of nor- and iso-alcohols of equal carbon number are compared,
\[ iC_3 < C_3, \quad iC_4 > C_4, \quad iC_5 < C_5 \]
The relation between C_4 and iC_4 is different from the result of OTTOSON (1958). Since the concentrations of the odorous compounds are not stated in his paper, it may not be proper to compare our results directly with his.

In acetate series,
\[ C_1 < C_2 < iC_3 < C_9 < C_4 < C_5 \leq iC_4 \leq iC_5 \]

When the stimulating effectiveness of nor- and iso-acetates are compared,
\[ C_8 > iC_3, \quad C_4 \leq iC_4, \quad C_5 \leq iC_5 \]

In ether series,
\[ C_6 < iC_5 \leq C_5 < C_2 < C_4 < iC_3 \leq C_3 \]

When the stimulating effectiveness of nor- and iso-ethers are compared.
\[ iC_3 \leq C_3, \quad iC_5 \leq C_5 \]

Since iC_4 ether was not available, comparison between iC_4 and C_4 was not possible.

The relation between stimulating effectiveness and chain length is most regular in acetate series, but is most irregular in alcohol series. Such an irregularity was also observed in the series of fatty acids by OTTOSON (1958). Though BACKMAN (1917) ascribed such irregularity to impurities in the test solution, other factors, such as the difference of physico-chemical properties of the substances may also be involved.

II. Comparison of the stimulating effectiveness of homologous substances by single application method. The saturated vapours of homologous alcohols (C_1 to C_8) were used as stimuli. The slow potential appearing in the olfactory epithelium increased its amplitude from C_1 to C_6, but decreased from C_6 to C_8 (Fig. 2). The amplitudes are plotted against the number of carbons in Fig. 3. A peak was found at C_6 and a break at C_4 in the curve. They show discontinuity in the increase of the stimulating effectiveness. It seems that the changes of the amplitudes of the
induced waves are nearly proportional to those of the slow potentials (Fig. 2). A reversed relationship was found in the changes of the amplitudes of the slow potentials between the normals and the isomers of C₃- to C₅-alcohols (Fig. 3).

OTTOSON (1958) performed a similar experiment, using alcohols dissolved in oil. There is found a considerable similarity between his and our curves. Only the difference is in the site of the peak. It is at C₆ in our case, while it is at C₇ in his case. It has been well known that the difference of the solvents results in different stimulating effectiveness (DETHIER, 1952, OTTOSON, 1958). This may be the cause of the difference in the sites of peaks.

OTTOSON (1958) studied the stimulating effectiveness of homologous alcohols which were dissolved in water at the concentrations of 0.001 M and 0.01 M. He
found that it increases nearly linearly as the series is ascended. This is very different from our result. It is known that water solubility decreases linearly for higher members of alcohols and it is very low at \( C_7 \) and \( C_8 \). Consequently, these high members of alcohols float on the water surface which make the intensities of these vapours relatively higher than those of the lower members of alcohols. This is supposed to be the origin of his different finding. In our experiment, saturated vapours of alcohols were used. Since vapour pressure decreases linearly as the series is ascended (DETHIER and YOST, 1952), the decrease of the stimulating effectiveness is conceivable for the higher members in our case.

The same experiment was repeated to study the stimulating effectiveness of acetates and ethers. In case of nor- and iso-acetates, only a general increase was found from \( C_1 \) to \( C_5 \) (Fig. 4). In case of ethers, a gradual increase from \( C_2 \) to \( C_3 \),
Fig. 5. The slow potentials (top) and the induced waves (bottom) produced by homologous nor-ethers. A short horizontal bar on the left below indicates the time and duration of stimulation. The summation of the on- and the off-slow potentials are observed in the top two records. The inset on the right shows the relation between the amplitudes of the slow potentials (ordinate) and the number of carbon (abscissa). The amplitudes are shown as percentage of the one at C3. Further explanation in the text.

and then a gradual decrease from C3 to C6 were found in the amplitudes of both potentials (Fig. 5).

An off-response is produced very often by the application of ethyl ether vapour (Takagi, Shibuya, Higashino and Arai, 1960). The response types of the olfactory epithelium were studied among homologous ethers (Takagi and Higashino, 1961). An “off” type was found in case of C2 and iC3, and an “on-off” type in case of C5 and C4. The inflections found on the rising phases of the slow potentials in Fig. 5 (in case of C2 and C3) indicate the summation of the on- and the off-slow potentials. In the inset of Fig. 5, the ordinate shows the amplitude
of the on- or the off-slow potential as well as that of the off-slow potential superimposed on the on-slow potential. Consequently, it may not be proper to compare this curve with the curves of the other two substances shown in Fig. 3 and 4.

**III. The relation between the concentrations of alcohols in aqueous solution and the amplitudes of the slow potentials.** The experiment by OTTOSON (1958) was repeated for comparison. The results were very different from his ones in that the stimulating effectiveness did not follow linearly the increase of the carbon number in our case. In case of 0.01 M aqueous alcohols, OTTOSON found a gradual increase of the stimulating effectiveness from C₁ to C₃ and then an abrupt linear increase from C₅ to C₆, while we found such an increase only from C₅ to C₆ and besides a gradual decrease from C₆ to C₈ (Fig. 6). In case of 0.001 M alcohols, OTTOSON found a linear increase only from C₆ to C₈, while we found a similar increase from C₅ to C₇ and a slight decrease from C₇ to C₈ (Fig. 6). In Fig. 6,

![Graph](image)

**Fig. 6.** Comparison of the stimulating effectiveness of homologous alcohols in aqueous solution of three different concentrations. Ordinate, the amplitudes of the slow potentials. Abscissa, the number of carbons. Further explanation in the text.

the curve for saturated alcohol vapour was added for comparison. The dependence of the stimulating effectiveness on the concentration is found only in the range from C₃ to C₆. The amplitudes of the slow potentials under or beyond this range are nearly the same in spite of the difference in concentrations. OTTOSON (1956) found and TAKAGI et al. (1961) confirmed that the slow potential of 80 to 90 µV is evoked even by stimulation with pure air. The slow potentials produced by 0.01 M and 0.001 M alcohols in the range of C₁ to C₄ (Fig. 6) can be evoked by stimulation with pure air, and the stimulating effectiveness of these weak alcohols may be negligible. This is supposed to be the reason why the difference of concentration does not have much influence on the slow potential at C₁ to C₄. On the other hand, water solubilities of C₇- and C₈-alcohols are so
small that most parts of these alcohols do not dissolve in water, but float on the water surface. The amounts of floating alcohols may not be much different and their vapour pressures are very weak (Ottoson, 1956). Considering these conditions, it is not surprising that the stimulating effectiveness are not much different at C7 from at C8, in spite of the change of concentrations from 0.001 M to saturation.

The difference of our result from Ottoson’s may be due to the difference in the properties of alcohols used in both laboratories, such as water solubility, oil solubility, and purity.

IV. The stimulating effectiveness of the homologous substances of equal thermodynamic activities. If molal free energy of a substance at a certain standard state which is in thermodynamic equilibrium is expressed by a certain value of thermodynamic activity, there is an experimental evidence that substances of equal thermodynamic activity have biologically equal stimulating effectiveness (Brink and Posternak, 1948). It was tested whether this rule is applicable for olfactory stimulation or not. The same experiment has already been performed by Ottoson (1958), but his data does not always seem to follow this rule.

According to Brink and Posternak (1948), thermodynamic activity $A$ in aqueous phase is calculated from the equation:

$$A = f_\infty N$$

where $f_\infty$: activity coefficient of a substance at infinite dilution.
$N$: mol fraction of the substance in solution.

Values of $f_\infty$ for alcohols at infinite dilution and at 25°C have been measured by Butler (1935) and were used in the calculation of the thermodynamic activity of narcotics by Brink and Posternak (1948). Ottoson (1958) found that the amplitudes of the slow potentials at C1 to C3 are considerably smaller than the standard one at C4. However, we found that they are nearly equal from C1 to C6 and only smaller at C7 and C8 (Fig. 7 A).

Then, the same experiment was performed in vapour phase according to the following equation (Brink and Posternak, 1948):

$$A = \frac{P_t}{P_s}$$

where $P_s$: saturated vapour pressure of a substance.
$P_t$: partial vapour pressure of the substance in equilibrium with the receptor cell.

The values of $P_s$ of homologous alcohols were taken from a table in Dethier and Yost’s paper (1952). The saturated alcohol vapours were diluted with pure air according to the values of $P_t$ which was calculated, when $A = 0.01$. The stimulating effectiveness were found to be nearly the same except at C6 and C7 (Fig. 7 B). The results of the above two experiments which were performed in different phases but at the same thermodynamic activity are nearly coincident except at C6 and C7.
In the present stage of our experiment, it is beyond our scope to clarify the mechanism of such disagreement at this region and also in the above two different phases. The same experiment was repeated, when $A=0.1$ in homologous alcohol solutions. The amplitudes were nearly similar except at $C_3$ and $C_5$ (Fig. 7 C).

When Fig. 7 C is compared with Fig. 7 A, it is found that the stimulating effectiveness are different considerably. All the curves were collected for comparison in Fig. 8. It is apparent that the curve is nearly even, when the value of $A$ is 0.01, but is becomes more uneven, when the value becomes bigger. The curve deviates most strikingly when saturated alcohol vapours were used, namely when $P_t=Ps$, hence $A=1$. Therefore, the value of thermodynamic activity must be under an upper limit ($A=0.1$ at most in our case) in order that Brink-Posternak's rule may be applied. On the contrary, when $A$ is very small, the stimulating effectiveness becomes too small to be discriminated from the response to pure air. Thus,
there is also a lower limit to the value of $A$. Therefore, it is apparent that their rule is applicable only in a small range of the thermodynamic activity for the olfactory epithelium of the frog.

The relation between the thermodynamic activity and the stimulating effectiveness can be studied only after an equilibrium state is attained between the odorous substance and the cell. When the thermodynamic activity becomes larger, it may take more time to attain an equilibrium state. Since the generation of the slow potential is transient, this may explain the larger deviation at the larger value of $A$ (Fig. 8).

When the value of the thermodynamic activity is 0.01 for acetates, the amplitudes of the slow potentials are nearly equal between C$_2$ and C$_6$ (Fig. 9.).
amplitude is smaller only at C1 than the others. However, the difference is minor and may be within the range of experimental error.

V. The stimulating effectiveness of homologous alcohols on the equimolecular basis. The saturated vapour pressure is lowest at C8 among the homologous alcohols. It was taken as a standard, and partial vapour pressures of the other alcohols were made equivalent to it, in order to make the number of molecules equal. The stimulating effectiveness was found to be largest at C6. In Fig. 10, the amplitudes of the other alcohols are shown in percentage of the one at C6. The values of the thermodynamic activities of these alcohols were calculated from the equation (2). It was found that they increase exponentially from C1 to C6 (Fig. 10). The

![Fig. 10](image-url)  
**FIG. 10.** The stimulating effectiveness of homologous alcohols on the equimolecular basis. Left ordinate, the thermodynamic activity. Right ordinate, the amplitude of the slow potential. Abscissa, the number of carbons. The effectiveness (broken line) increases from C1 to C6 and then decreases. The curve of the thermodynamic activity (continuous line) coincides well with the above curve in the range between C1 and C6. The deviation of the former curve from the latter one is explained in the text.

curve of the activities coincides well with that of the stimulating effectiveness in the range from C4 to C6, but it deviates in the lower and higher alcohols. As stated before the slow potential of 80 to 90 μV is evoked by stimulation with pure air. If these amplitudes are subtracted, the residual amplitudes at C1 to C3 become nearly zero, and the two curves in Fig. 10 coincides well. Therefore, on the basis of equimolecular reaction the activity A has a linear relationship with the stimulating effectiveness in the range from C1 to C6. Now, the curve of the stimulating effectiveness entirely deviates from the one of thermodynamic activity at C7 and C8. As shown before, the BRINK-POSTERNAK’s rule is not applicable, when the activity A is big enough. Since the saturated vapour was used at C8, the value of A is as big
as 1. This, together with the negligible water solubility may explain such a deviation at C7 and C8.

DISCUSSION

On the peak in the curve of the stimulating effectiveness. In Fig. 3, it was shown that the stimulating effectiveness of saturated vapours of homologous alcohols increases from C1 to C6 and then decreases for the higher members. In Fig. 10, a very similar relation is found between the stimulating effectiveness of equimolecular alcohols and the number of carbon atoms. In both cases, a peak is found at C6. A substance must be water and/or oil-soluble, so that it may stimulate the olfactory receptor and produce odorous sensation (Moncrieff, F 1944). C1- and C2-alcohols are infinitely soluble in water, but are almost insoluble in oil. Conversely, C7 and higher alcohols are infinitely soluble in oil, but practically insoluble in water. Therefore, the receptor can only be stimulated either with water-soluble component or oil-soluble one in these alcohols. Consequently, the slow potentials produced by either component may have relatively small amplitudes. On the contrary, alcohols in the range of C3 to C6 are water- and oil-soluble, which makes their stimulating effectiveness summative. This may explain why the slow potentials are bigger in the alcohols of C3 to C6 than in the others (Fig. 8). Dethier and Chadwick (1950) showed that the stimulating effectiveness of a substance is inversely proportional to the water solubility in the tarsal chemoreceptor of the blowfly. The peak of the curve may be produced at C6 by this and the above mechanisms. Dethier (1951) proposed a hypothesis that the limiting mechanism in contact chemoreception involves a two phase system whereby highly water-soluble compounds gain access to the receptor through an aqueous phase and the larger lipid-soluble compounds chiefly through a lipid phase. Our results entirely support his hypothesis.

Studying the thresholds of odorous substances in man, Mullins (1955) found that the olfactory stimulating powers of alcohols increase from C1 to C5, but decrease for higher members. Though the site of peak is different by one atom, it is very significant that our result in the frog coincides well with his one in man.

On the break in the curve of stimulating effectiveness. In Fig. 3, a break is found at C4 in the curve which relates the stimulating effectiveness to chain length. The same phenomenon has already been found by other workers. Chadwick and Dethier (1949) and Dethier (1951) found it at or near butanol (C8) in the tarsal receptor of the blowfly. Such a break was found only when water is employed as a solvent, but never when glycol or oil was employed. Therefore, Dethier offered this as evidence to support his hypothesis. Ottoson (1958) also found such a break at C9 in the homologous carboxylic acids. Since infinite
water solubility is lost in this region, he supposed that such a break is produced by an abrupt change of water solubility. Though the point of the break is different by one atom, the same explanation may also be applied in our case. DETHIER (1952) and OTTOSON (1958) found that the results become different when different solvents are used. This, together with the difference in other points may be the origin of minor differences between OTTOSON’s and our results.

On the relation between the steric configuration and the stimulating effectiveness. The relation between the steric configuration of a molecule and its olfactory stimulating effectiveness has been studied by several workers. DETHIER and CHADWICK (1950) found that branched hydrocarbons have less stimulating effect that straight hydrocarbons on the tarsal receptors of insects. OTTOSON (1958) found the same relation in the olfactory epithelium of the frog between n-butanol and isobutanol. In our experiments, it was found that the compounds with straight hydrocarbons do not always have more stimulating effect than those with branched hydrocarbons (FIG. 3). Our research work, though scanty, does not always support the finding by DETHIER and CHADWICK (1950).

SUMMARY

1. The stimulating effectiveness of homologous series of alcohols, acetates and ethers were studied in the olfactory epithelium of the frog by successive and single application methods. The results obtained by both methods were entirely coincident.

   The stimulating effectiveness of a saturated alcohol vapour increases following the increase of carbon number, but it decreases beyond C₆. The stimulating effectiveness of a saturated acetate vapour increases gradually as the carbon number increases. But that of ether vapour has a maximum at C₃, and it decreases as the carbon number increases.

2. The stimulating effectiveness of alcohol vapour from the aqueous solution was studied at the concentration of 0.001 M and 0.01 M. The results were different from those obtained by OTTOSON.

3. The stimulating effectiveness was studied in the homologous substances of equal thermodynamic activities. It was found that they are nearly the same when the value of the thermodynamic activity is 0.01, but that they become different when the value exceeds it. It was made clear that BRINK-POSTERNAK’s rule (1958) is only applicable in a small range of the values of the thermodynamic activity in the frog.

4. The stimulating effectiveness of alcohols was studied on the equimolecular basis. The results were coincident with the calculated values of the thermodynamic activities of these alcohols except at C₇ and C₈. The mechanism of such a deviation was considered.
5. The relation between the steric configuration of the odorous substance and the stimulating effectiveness was studied.

6. There were found a peak and a break in the curve which shows the stimulating effectiveness of homologous alcohols. The origins of these discontinuities were discussed.

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