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# Hydrophobicity of Biosurfaces as Shown by Chemoreceptive Thresholds in *Tetrahymena, Physarum* and *Nitella*

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*Summary.* Responses (chemotaxis and changes in membrane potential) of *Tetrahymena, Physarum,* and *Nitella* against aqueous solution of homologous series of *n*-alcohols, *n*-aldehydes and *n*-fatty acids were studied for clarifying the hydrophobic character of chemoreceptive membranes. Results were: (1) All organisms studied responded to homologous compounds examined when the concentration of these chemicals exceeded their respective threshold, $C_{th}$, and the response, $R$, were expressed approximately as $R=\alpha \log (C/C_{th})$ for $C>C_{th}$. (2) Increase of the length of hydrocarbon chain in homologues decreased $C_{th}$. Plots of $\log C_{th}$ against the number of carbon atoms, $n$, in *n*-alcohols, *n*-aldehydes and *n*-fatty acids showed linear relationships as represented by $\log C_{th}=-An+B$. $A$ and $B$ are positive constants for respective functional end groups of the chemicals and biological membranes used. The above empirical equation was interpreted in terms of the partition equilibrium of methylene groups between bulk solution and membrane phase. Parameter $A$ was shown to be a measure of hydrophobicity of the membrane, and $B$ represented the sensitivity of chemoreception of the membrane. (3) Thresholds, $C_{th}$, for various hydrophobic reagents were compared with those of human olfactory reception, $T$. Plots of $\log T$ against $\log C_{th}$ fell on straight lines for respective organisms with different slopes which were proportional to parameter $A$.

Interaction of chemicals with biological membranes brings about important versatile functions such as taste and olfactory receptions, synaptic junctions, hormonal actions, narcotics, hemolysis, etc. Many investigators in the field of chemoreception concentrated their attention on finding specific molecules (proteins) which bind preferentially certain chemicals. This kind of research was successful in finding receptor proteins of acetylcholine [13, 24, 27], sugars [1, 2, 6, 14, 15, 17, 22], etc. On the other hand, it is well known that there is no specific receptor site in anesthetics, i.e., chemicals which have higher affinity with oils are more effective in narcotics [16, 28]. Responses of living membranes to so-called odorous substances also seem to belong to the latter category.

Clues to this understanding come from the facts that responses to hydrophobic chemicals were observed widely in nature from bacteria

to sensory organs of higher vertebrates, and that the effective power of stimulation in olfactory response showed a close relationship with the solubility of odorants against oils [4, 8, 12, 23, 26, 30, 31, 32]. Little systematic study, however, has been performed along this line of thought. In addition, physiological studies on sensory responses in vertebrates are difficult in general and require skillful techniques, because the sensory cells are generally too small to investigate the physicochemical processes in chemoreception at membrane level.

Considering these situations, we are tempted to study responses to various chemicals by using giant cells as model systems of chemoreception. We have chosen *Nitella* internodes, *Tetrahymena* and *Physarum*. By using *Nitella*, we can obtain information of chemoreception at the receptor membrane level because the giant excitable cell of *Nitella* permits us intracellular measurement of membrane potential during chemoreception. Ciliate protozoan *Tetrahymena* exhibits positive or negative chemotactic response, which may be considered as a primitive form of discrimination. Furthermore, biochemical studies on *Tetrahymena* have been so extensively performed that physiological data obtained here will possibly be related to biochemical events in future study [18]. The protoplasmic streaming of *Physarum polycephalum* is one of the best understood motile systems so far studied among lower organisms [19, 20]. Therefore, *Physarum* is suitable for the study of transduction mechanism of the information sensed at the receptor membrane to motile system. Last but not least, comparison is made between different types of organisms: plants and animals, protozoa and higher vertebrates, ciliary and amoeboid movements, etc.

In this paper we will show, first, that response to hydrophobic reagents is a common phenomenon among various biological species and, second, that response to homologous compounds of *n*-alcohols, *n*-aldehydes and *n*-fatty acids can be analyzed thermodynamically in terms of partition equilibrium of the chemicals between bulk solution and membrane phase. The same analysis will be applied to olfactory reception in higher vertebrates and insects, comparison being performed between various living organisms.

## Materials and Methods

### *Measurements of Chemotaxis in Slime Mold Physarum polycephalum*

*Physarum polycephalum* (a gift from Prof. N. Kamiya, Osaka University, Osaka) was cultured by the method employed by Camp [5]. The plasmodium was allowed to crawl

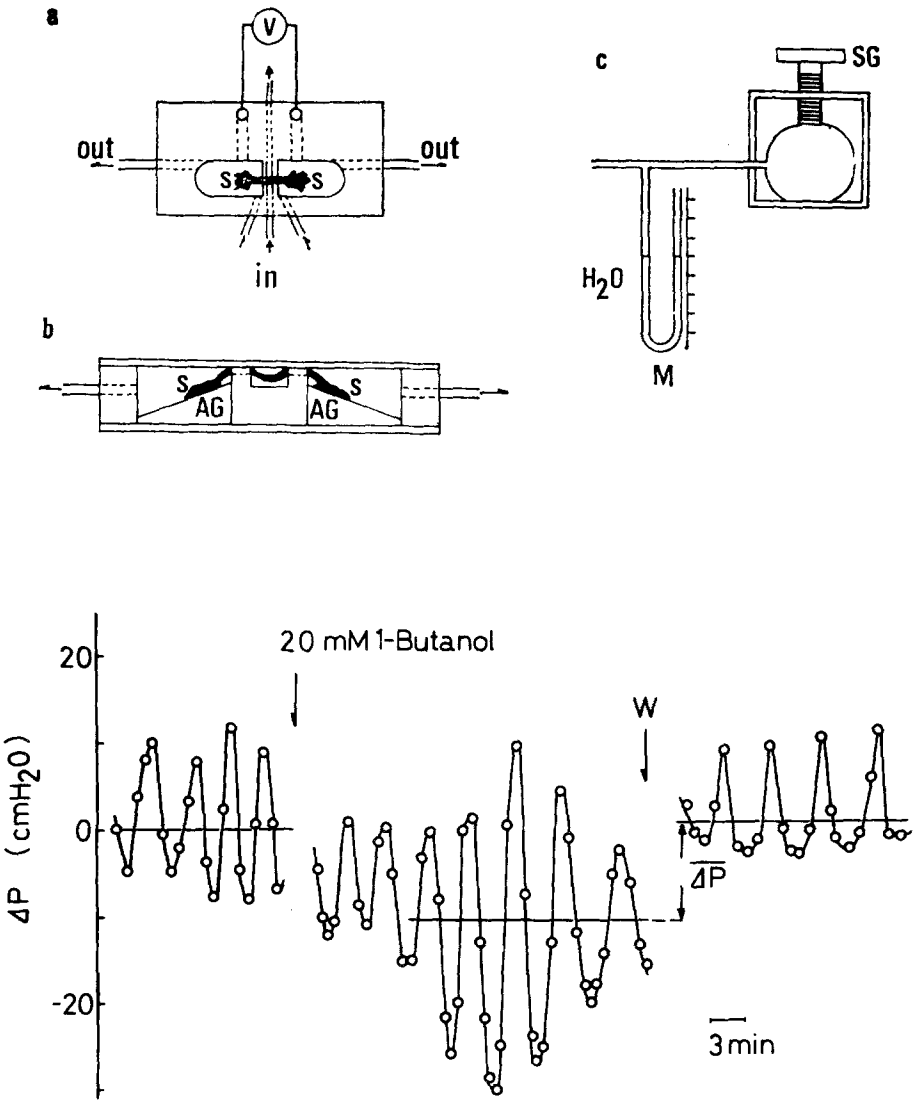


Fig. 1. Schematic diagram illustrating the experimental setup for measuring the motive force of slime molds. (*a*) and (*b*): top and side views of the double chamber. (*c*): apparatus supplying the pressure difference. Lower half: Effect of 1-butanol on plasmodynamogram of plasmodium *Physarum polycephalum*. *W*: time of removal of the chemical stimulus

overnight with no feeding on 0.7% agar gel before use. Chemotactic motive force was measured by applying the double chamber method proposed by Kamiya [21]. Experimental details were described in a previous paper [34]. The method is based on the fact that the protoplasmic streaming can be controlled by applying a difference in hydrostatic pressure, $\Delta P$, between two compartments (*see* Fig. 1). The lower half of Fig. 1 shows an example

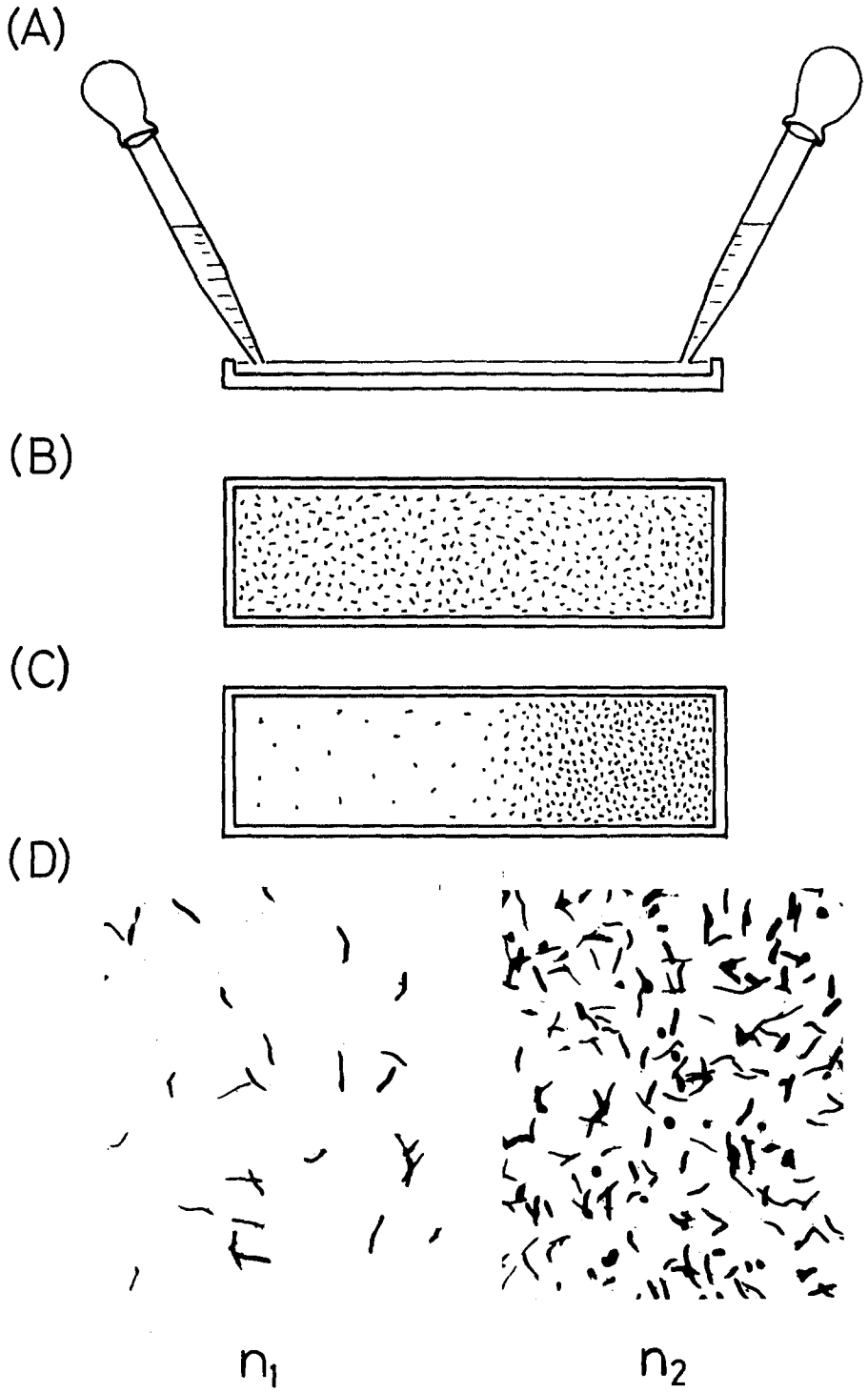


Fig. 2

of dynamoplasmogram, where 20 mM of 1-butanol was applied to one compartment of the double chamber. Motive force deviated $\overline{\Delta P}$ *on the average and returned to the original level with removal of chemical stimulus.* $\overline{\Delta P}$ affords a quantitative measure of chemotactic movement in plasmodium of *Physarum polycephalum* and is referred to as the chemotactic motive force.

### *Measurements of Chemotaxis in Tetrahymena*

*Tetrahymena pyriformis* was kindly furnished by Dr. T. Mita, National Institute of Cancer Research, Tokyo. *Tetrahymena* was cultured at 20–25 °C in a solution containing 2% proteose peptone, 1% yeast extracts and 0.6% glucose. The organisms were washed centrifugally (2 min under $300 \times g$) with distilled water several times and stored for use. In this condition *Tetrahymena* survived more than 3 days without feeding. Just after centrifugation, some of *Tetrahymena* swam irregularly or slowly. To avoid ill effects of centrifugation, most of the experiments were performed between 10 and 20 hr after washing. Fig. 2 shows a schematic diagram of the experimental arrangement and procedures. Chemotaxis was observed in a thin glass vessel ($2 \times 6$ cm wide, 1 mm thick). One side of the vessel was filled with test solution (i.e., a given amount of a chemical was dissolved in distilled water containing *Tetrahymena*) and the other side with distilled water containing an equal number of the organisms. *Tetrahymena* was, therefore, distributed uniformly at the beginning of experiment (*see* Fig. 2B). As time went on, *Tetrahymena* gathered to one side when the concentration of the chemical in the test solution exceeded a certain value. After 20 to 30 min, steady distribution of the organisms was attained, as seen in Fig. 2C. The number of organisms was counted by taking photographs microscopically ($\times 10$ magnification) with 1 sec exposure under dark-field illumination. Duration of illumination must be as short as possible, because the organisms move away from the light. Fig. 2D shows photographs obtained in this way, which indicates the locus of the movement of *Tetrahymena*. By counting the number of organism $n_1$ and $n_2$ in test solutions and in water per unit area, respectively, we can obtain the fraction of responding organisms, $R$, by the following equation:

$$R=(n_1-n_2)/(n_1+n_2)$$

which expresses chemotactic response in *Terahymena* quantitatively. Positive and negative signs of $R$ indicate positive and negative taxis, respectively.

Analyzing locus of the movement, we will also obtain information about the mechanism of movement of the organisms toward or away from the chemical stimuli. We can mention here that changes both in migration velocity and in tumbing frequency are responsible for the chemotactic movement of *Tetrahymena*. In a solution of repellent, organisms move faster than in distilled water, or vice versa for attractant. Tumbling frequency increased as the organisms move toward more concentrated repellent solution. Details of these observations will be reported elsewhere.

Fig. 2. Schematic illustration of apparatus and procedure for measuring chemotaxis in *Tetrahymena*.(*A*) Equal volumes of test and control solutions containing equal number of organisms were put in thin vessel. (*B*) Uniform distribution of the organisms at time zero. (*C*) Distribution of the organisms after 30 min (the steady distribution). (*D*) Photographs of each side of the vessel taken with 1 sec exposure. $n_1$ and $n_2$ are number of organisms per unit area in test solution and distilled water, respectively

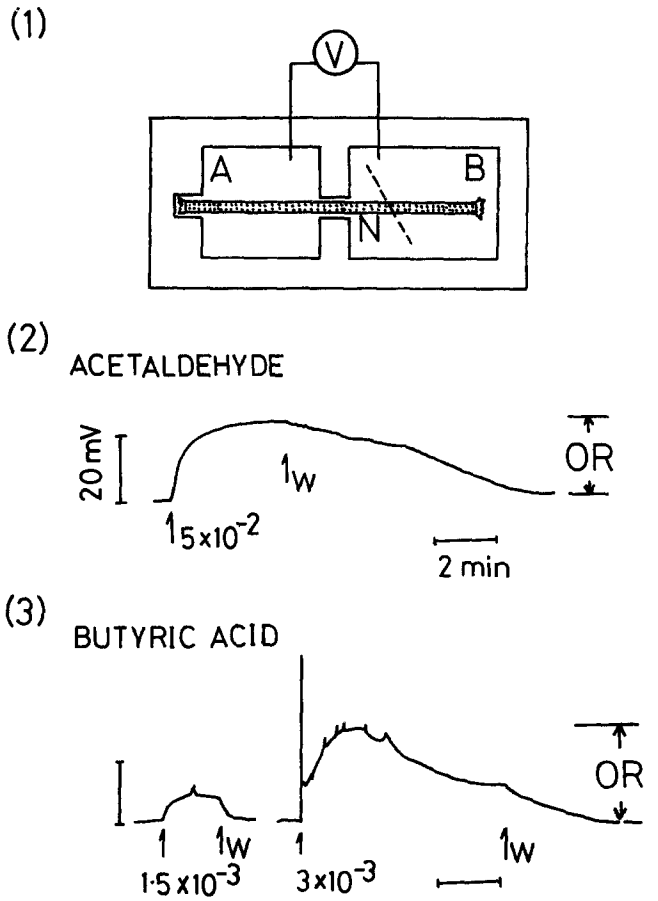


Fig. 3. Potential response of *Nitella* internode to acetaldehyde and butyric acid. (*1*) Experimental setup of potential response measurement by means of open vacuole method [29, 31]. (*2*) Changes in membrane potential by application of 50 mM acetaldehyde. (*3*) Response of *Nitella* to 1.5 mM and 3 mM of butyric acid. *W*: time of removal of chemical stimuli

*Electrical Response of Nitella Internode to Chemicals*

*Nitella sp.* used were collected from Lake Utonai, Hokkaido, during the summer of 1975 and were cultured in tap water at 25 °C under illumination of 40 W fluorescent tubes at 12-hr intervals. A giant internodal cell was separated from *Nitella* and was stored in artificial pond water (0.025 mM KCl, 0.05 mM NaCl, 0.05 mM $NaH_2PO_4$, 0.2 mM $Ca(NO_3)_2$ and 0.1 mM $MgSO_4$) over three days.

Membrane potential was measured intracellularly by applying the open-vacuole method [29]. Experimental details were described in a previous paper [31]. An internodal cell was separated electrically by vaseline into two portions (*see* Fig. 3(*1*). One portion of the cell was amputated under isotonic condition. Chemicals were applied to the other portion of the internodal cell in compartment *A* in Fig. 3(*1*) as an aqueous solution dissolved in 0.5 mM $CaCl_2$, where the solution was kept isotonic by adding 0.3 M mannitol. The potential response caused by application of the chemical was measured between compartments *A* and *B*. Fig. 3(*2*) and (*3*) show typical examples of potential responses of *Nitella*

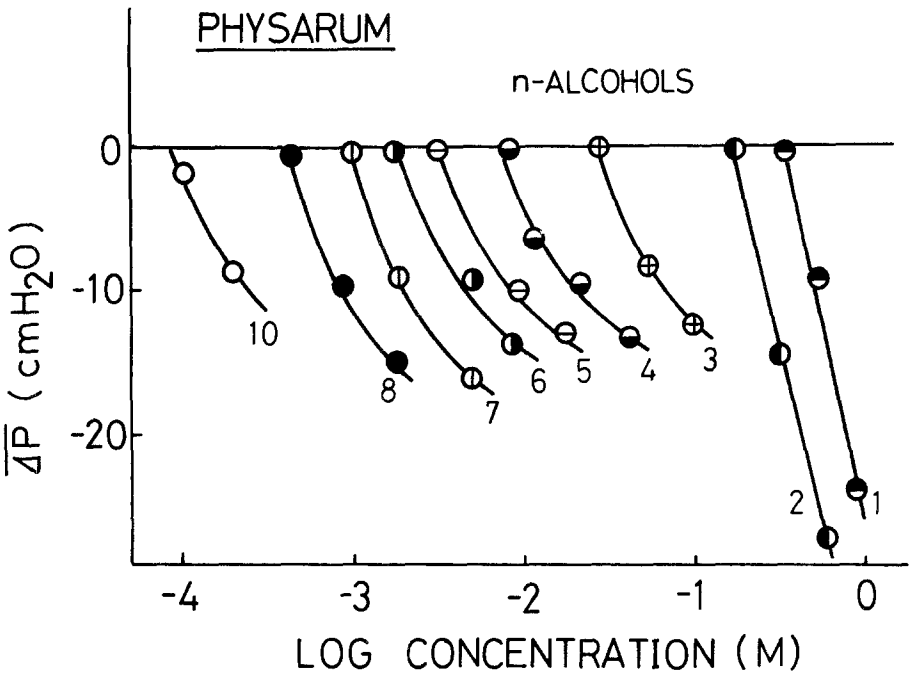


Fig. 4. Concentration dependence of chemotactic motive force, $\Delta P$, in *Physarum* against *n*-alcohols. Numbers in the figure denote the number of carbon atoms in *n*-alcohols

when solutions of acetaldehyde and butyric acid were applied to an internode. Application of 50 mM of acetalehyde gradually depolarized by 25 mV and removal of it slowly recovered the initial level of the membrane potential. Different patterns of the potential variations were observed for butyric acid, depending on the concentrations applied. 1.5 mM of 1-butyric acid induced only slow response, as observed with acetaldehyde, but 3 mM of 1-butyric acid induced an action potential which followed the gradual change in membrane potential whose maximum depended on the concentration applied. Note that small spike-wise changes in membrane potential were superimposed on gradual depolarization. We take the magnitude of slow response as the potential response of *Nitella* to chemicals and denote it as *OR* in this article.

All experiments were performed at room temperature $21 \pm 1$ °C. To confirm the reproducibility of the experimental results, data were taken four times at a given condition.

### *Chemicals*

Homologous series of *n*-alkyl alcohols, aldehydes and fatty acids were used as chemical stimuli. These chemicals of analytical grade were used as delivered. Water used as solvent was distilled twice in glass vessels.

## Results

### *Responses of Physarum to n-alcohols and n-aldehydes*

Fig. 4 shows chemotactic motive force, $\overline{\Delta P}$, against logarithm of concentration ($C$ in moles/liter) of series of alcohols applied, where the

number of carbon atoms in *n*-alcohols is denoted in the figure. The similar $\overline{\Delta P}$ *vs.* log $C$ relationships are obtained for *n*-aldehydes. No response was observed below respective threshold concentration, $C_{th}$, and above $C_{th}$ the plasmodium began to move away from *n*-alcohol and *n*-aldehyde solutions. $\overline{\Delta P}$ increased more than 10 cm $H_2O$ as the concentration increased. This fact contrasts with the case of sugar or salt receptions, where $\overline{\Delta P}$ changed in almost all-or-nothing fashion at $C_{th}$ by $\pm 10$ cm $H_2O$ and remained at constant level with further increase in concentration [34]. Increase in number of methylene group in *n*-alcohols and *n*-aldehydes decreased $C_{th}$ systematically, as seen in Fig. 4. Large changes in $\overline{\Delta P}$ for methanol and ethanol may be attributed to the osmotic effect, as reported previously [33]. Membrane potential depolarized by 5 to 10 mV when the concentration exceeded $C_{th}$. $C_{th}$ determined from the membrane potential agreed with that of $\overline{\Delta P}$. This agreement of $C_{th}$ in chemotactic movement and potential change was already reported for the case of sugar and salt receptions [34].

### *Chemotaxis in Tetrahymena against n-alcohols, n-aldehydes and n-fatty Acids*

The upper trace of Fig. 5 shows the concentration dependence of chemotactic response of *Tetrahymena* for *n*-alcohols and *n*-aldehydes. The number of carbon atoms is denoted in the figure at the bottom of the respective curves. As in the case of slime mold, *Tetrahymena* did not react to alcohols and aldehydes until the concentration of chemicals exceeded their respective threshold values. Above $C_{th}$, the number of reacted organisms increased linearly with log $C$.

For fatty acids, change in pH of the stimulus solution became appreciable at high concentration region. Therefore, the value of pH was adjusted to 5.5 with 1 mM phosphate buffer, in this case. The lower trace in Fig. 5 shows concentration dependence of chemotactic response in *Tetrahymena* against *n*-fatty acids. *Tetrahymena* showed a negative chemotaxis against *n*-fatty acids, in general. Contrary to alcohols and aldehydes, however, some fatty acids attracted *Tetrahymena* in a certain range of concentration. Even in these cases, the organisms showed negative response when the concentration became high. Butyric acid, especially, is specific because the positive taxis is induced by a concentration as low as $10^{-7}$ M, and about half of the organisms are attracted to the chemical until $10^{-4}$ M. Further increase of concentration led to a negative taxis for butyric acid. This low value for positive attractant for butyric acid may be important for *Tetrahymena* to capture prey organisms in the

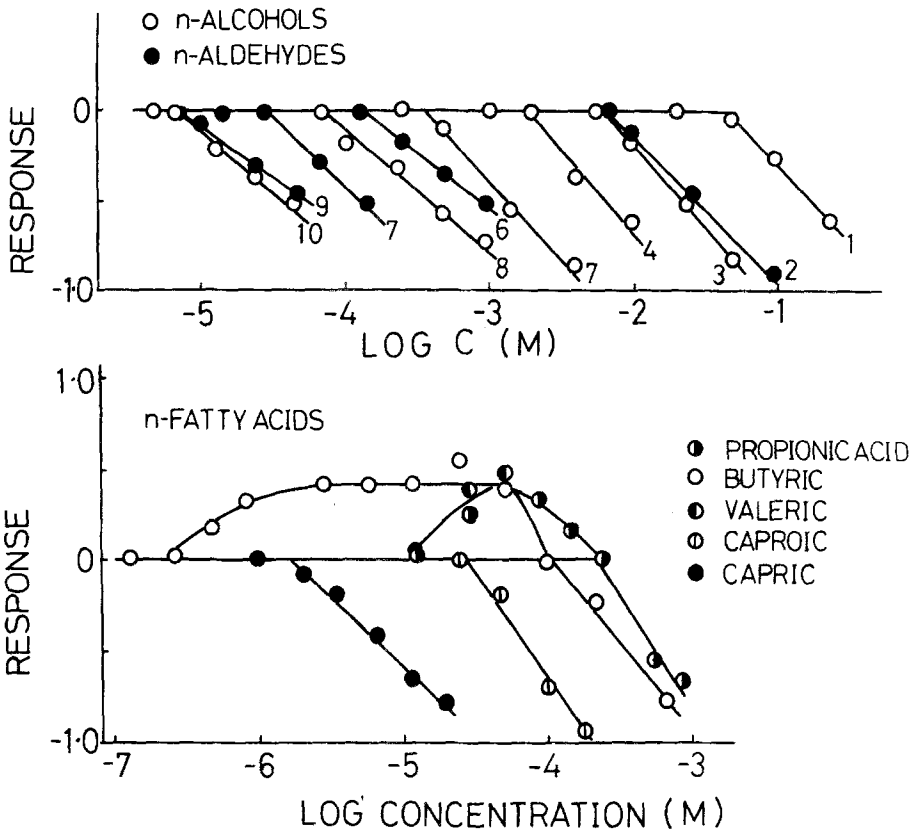


Fig. 5. Concentration dependence of chemotactic response in *Tetrahymena* against *n*-alcohols, *n*-aldehydes and *n*-fatty acids. Upper trace: ○: *n*-alcohols; ●: *n*-aldehydes. Number of carbon atoms in homologues is denoted in the figure. Lower trace: *n*-fatty acids

natural environment, because butyric acid is one of the common excretion substances in bacteria. An increase or a decrease of methylene group from butyric acid, i.e., valeric and propionic acids, respectively, decreased remarkably the positive response, as compared with butyric acid. In this respect, we may say that butyric acid is a specific fatty acid in chemotaxis of *Tetrahymena*. Even for these fatty acids, *Tetrahymena* moved away from the chemicals when the concentration exceeded their respective threshold values. Therefore, the critical concentration of negative taxis is referred to as the threshold of the fatty acids and is denoted by $C_{th}$ as before.

### *Response of Nitella Internodes to n-alcohols, n-aldehydes and n-fatty Acids*

Fig. 6 shows the variation of intracellular potential, *OR* (*see* Fig. 3), when the solutions of homologues of *n*-alcohols and *n*-aldehydes were

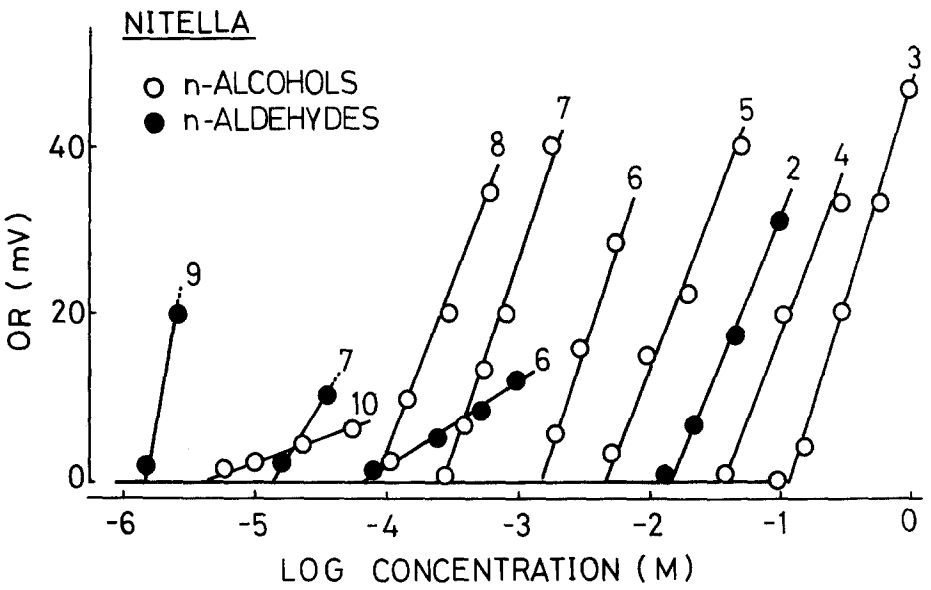


Fig. 6. Responses of *Nitella* internode against *n*-alcohols and *n*-aldehydes as a function of concentration of chemical stimuli. ○: *n*-alcohols; ●: *n*-aldehydes. Numbers of carbon atoms in each homologue are denoted in the figure

applied to the internodal cell of *Nitella*. Here, *OR* is plotted against logarithm of concentration of *n*-alcohols (open circles) and *n*-aldehydes (closed circles), respectively, and the number of carbon atoms is indicated in the figure. Some of the data are taken from the previous paper [31]. In all cases, membrane potential did not change appreciably below $C_{th}$ for respective chemicals, and above $C_{th}$ membrane potential depolarized linearly with log *C*. As seen in the figure, *n*-alcohols with n of 3 to 8 induced large depolarization of membrane potential, while decanol (n = 10) caused small responses. As reported in the previous paper [32], application of *n*-alcohols evoked an all-or-nothing type action potential which followed a slow depolarization of the potential just like the case of butyric acid shown in Fig. 3(*3*). This response pattern was seen for *n*-alcohols of n = 3 to 8, but decanol did not evoke the initial action potential. Acetaldehyde induced a large depolarization of membrane potential when the concentration exceeded its threshold. Hexylaldehyde caused small change of membrane potential. For heptyl- or nonylaldehyde, production of an action potential was often accompanied with an irreversible depolarization of membrane potential as denoted by dotted lines in the figure. Response pattern of butyric acid was as shown in Fig. 3(*3*), while heptanoic acid suppressed the excitability, i.e., no initial action potential was induced. These observations indicate that response

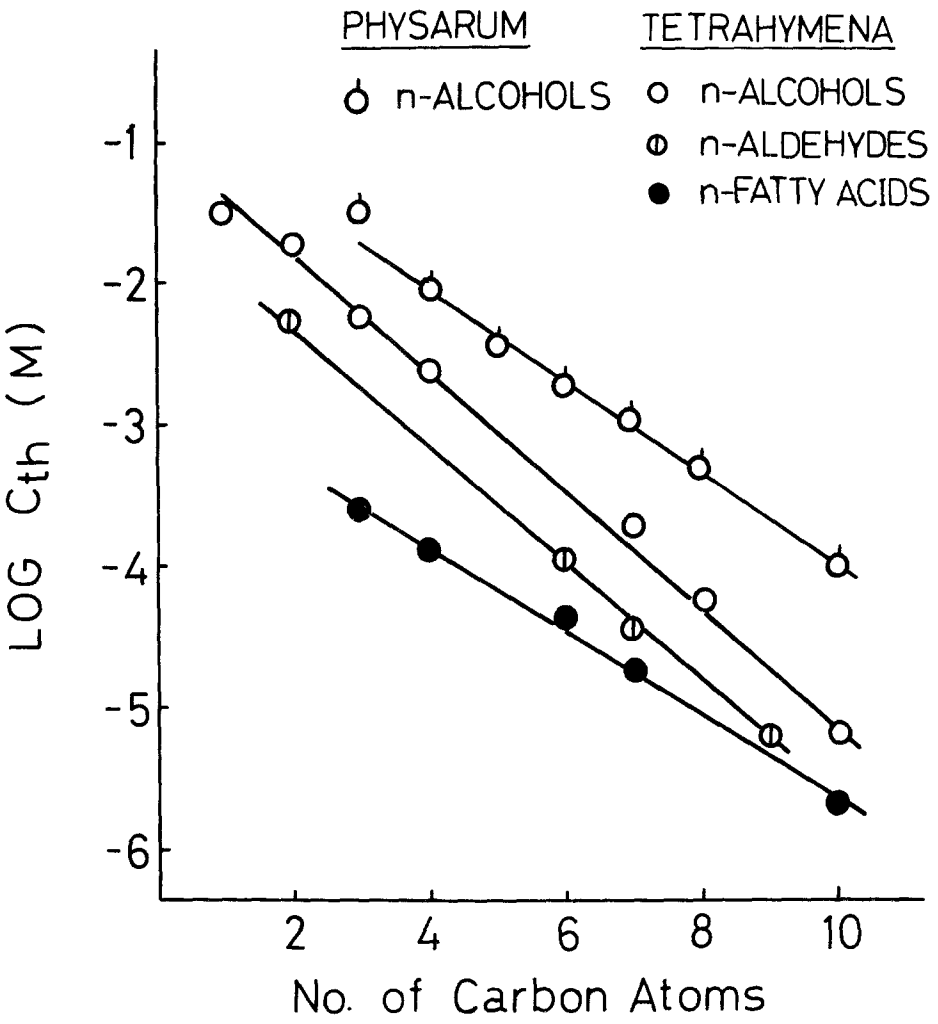


Fig. 7. Dependence of response thresholds on chain length of homologous compounds in *Physarum* and *Tetrahymena*. log $C_{th}$ is plotted against number of carbon atoms in the compounds. ♁: *n*-alcohols for *Physarum*; ○: *n*-alcohols; ⊕: *n*-aldehydes; ●: *n*-fatty acids for *Tetrahymena*

pattern varies from one chemical to another, which implies a discrimination of chemicals at the membrane level.

### *Dependence of $C_{th}$ on the Chain Length of Alkyl Homologues*

Fig. 7 shows plots of log $C_{th}$ for *Tetrahymena* and *Physarum* against the number of carbon atoms, $n$, of alcohols, aldehydes and fatty acids, where data for $C_{th}$ were taken from Figs. 4 and 5. The data show that

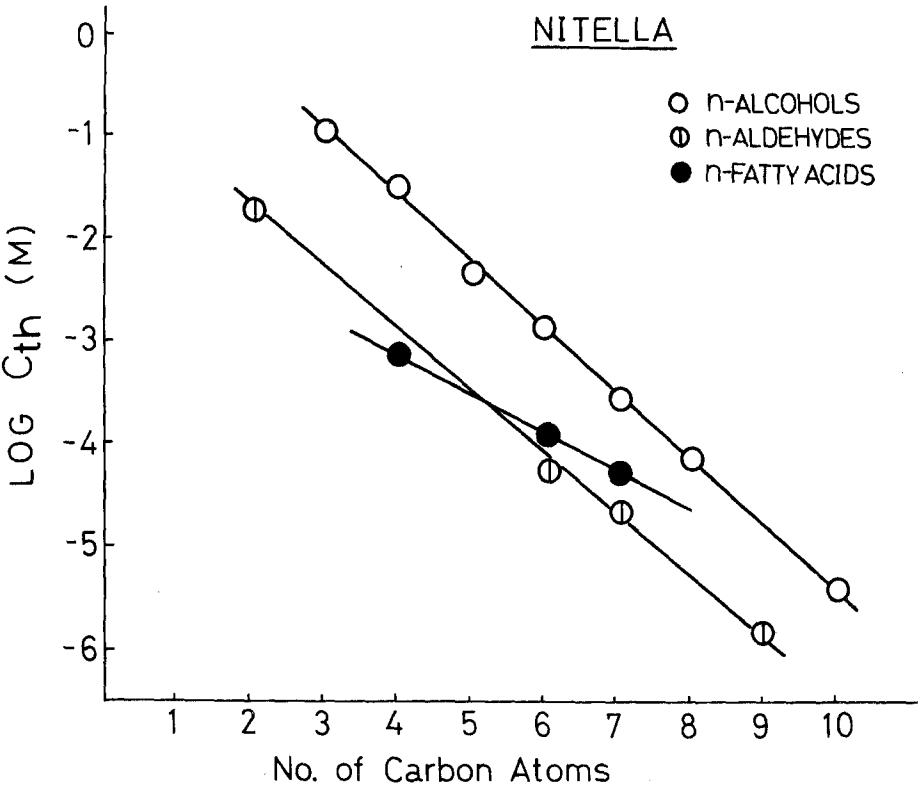


Fig. 8. Dependence of response thresholds of *Nitella* on the chain length of *n*-alcohols, *n*-aldehydes, and *n*-fatty acids, where log $C_{th}$ is plotted against the number of carbon atoms in the compounds. ○: *n*-alcohols; ⊖: *n*-aldehydes; ●: *n*-fatty acids

linear relationships hold between log $C_{th}$ and $n$ for each homologue and that the slopes of the straight lines depended on the functional end groups of the compounds and also on the biological membranes. Among functional groups examined, fatty acids depended slightly on the length of hydrocarbon chains, as compared with alcohols and aldehydes. For alcohols, $C_{th}$ for *Physarum* depended little on the length of hydrocarbon chains, as compared with that for *Tetrahymena*.

Notwithstanding complicated response patterns in *Nitella* (*see* Fig. 3), the threshold for homologous compounds examined vary systematically. Figure 8 shows the linear relations between log $C_{th}$ and $n$ for *Nitella*, slopes of which differ for different functional end groups.

To summarize, the effects of chain length in homologous compounds are expressed by the following equation:

$$\log C_{th} = -A\,n + B \tag{1}$$

where $A$ and $B$ are numerical constants having different values for different functional groups and membranes. Physicochemical implication of Eq.(1) will be considered subsequently.

## Discussion

### *Physicochemical Meaning of Empirical Equation (1)*

Eq.(1) can be derived from the thermodynamical analysis of the partition equilibrium of chemicals between aqueous and membrane phases. The chemical potential of a reagent in the bulk solution and membrane phase are denoted by $\mu_i^s$ and $\mu_i^m$, respectively. At equilibrium, chemical potential of $i$ in two phases must be equal each other. Hence we have

$$\mu_i^{0s} + RT \ln C_i^s = \mu_i^{0m} + RT \ln C_i^m \tag{2}$$

where $\mu_i^{0s}$ and $\mu_i^{0m}$ stand for the standard chemical potentials, and $C_i^s$ and $C_i^m$ for concentrations of the chemical in the bulk solution and membrane phase, respectively. Rearrangement of Eq. (2) with the assumption that difference in the standard chemical potential is proportional to the number of methylene groups, $n$, leads to

$$\ln C_i^s = -(\mu_{CH_2}^{0s} - \mu_{CH_2}^{0m})\, n/RT + \ln C_i^m. \tag{3}$$

The assumption used in the derivation of Eq.(3) has been confirmed in various nonbiological systems such as oil/water partition equilibrium [7, 16].

Comparison of Eqs.(1) and (3) gives physicochemical interpretation of parameters $A$ and $B$:

$$A = (\mu_{CH_2}^{0s} - \mu_{CH_2}^{0m})/2.3\ RT \tag{4}$$

$$B = \log C_i^m + \text{constant}. \tag{5}$$

Eq.(4) states that parameter $A$ is proportional to the difference in the standard chemical potential of a methylene group between bulk solution and membrane phase. Since the hydrocarbon chain is a typical hydrophobic substance, value of $A$ affords a quantitative measure of hydrophobicity of the membrane. The larger the value of $A$ the more hydrophobic the biological membrane. Parameter $B$ expresses the sensitivity of the membrane, as will be discussed below.

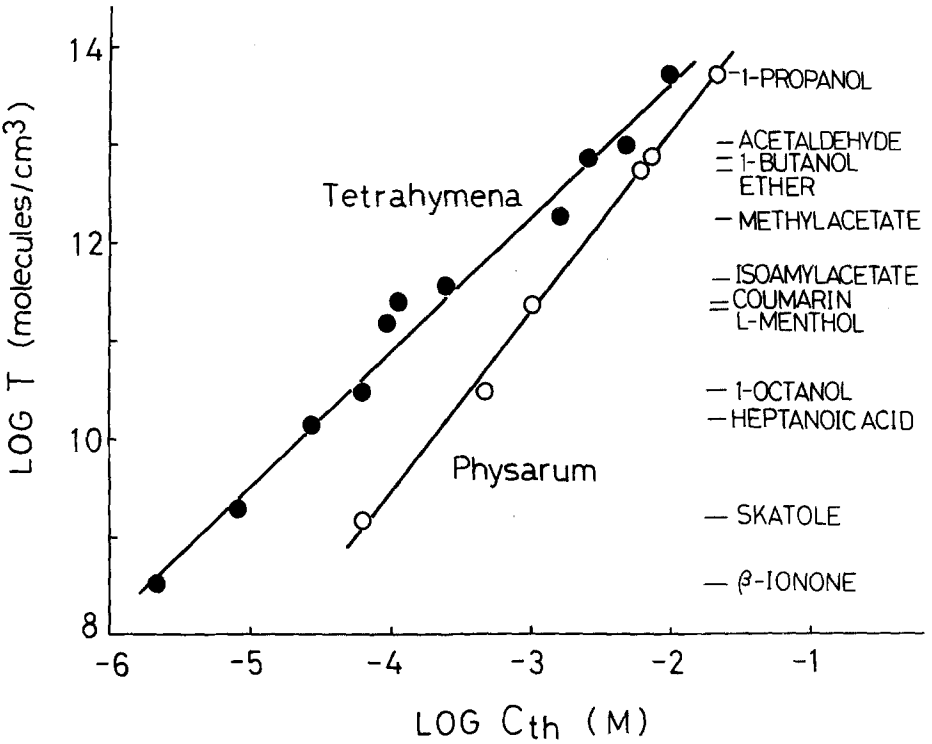


Fig. 9. Relationship between olfactory threshold of human, $T$, and chemotactic thresholds, $C_{th}$, of *Tetrahymena* and *Physarum*

*Comparison of the Chemoreceptive Thresholds in Tetrahymena and Physarum with Olfactory Threshold in Human*

Analysis described above has been applied in the field of anesthetics [16], but along this line of thought, little attention has been paid to the olfactory reception in higher animals. Fig. 9 compares olfactory threshold of the human [23], $T$, with those of *Tetrahymena* (closed circles) and *Physarum* (open circles), where thresholds are plotted in double logarithmic scales. $C_{th}$ of *Tetrahymena* and *Physarum*, other than homologous compounds described above, were determined by plots similar to Figs. 4 and 5. For various odorants denoted in Fig. 9, linear relationships hold for both *Tetrahymena* and *Physarum*, slopes being different between the two. Slopes of log $T$ *vs.* log $C_{th}$ plots are about 1.5 and 2.0 for *Tetrahymena* and *Physarum*, respectively.

Linear relationships between log $T$ and log $C_{th}$ also held for potential response in *Nitella* with unit slope [31], surface pressure increase in

Table 1

| Species | $\Delta\mu_{CH_2}^0$ (OH) (kcal/mole) | $\Delta\mu_{CH_2}^0$ (CHO) (kcal/mole) | $\Delta\mu_{CH_2}^0$(COOH) in kcal/mole | Ref. |
|---|---|---|---|---|
| Man | 0.95 (1–8) | | | [7] |
| Rat | 0.9 (1–10) | | | [25] |
| Blowfly | 0.71 (1–10) | 0.72 (3–8) | | [10, 11] |
| Nitella | 0.87 (3–8) | 0.84 (2–9) | 0.45 (4–7) | |
| Tetrahymena | 0.73 (1–10) | 0.73 (2–9) | 0.39 (3–10) | |
| Paramecium | 0.71 (1–5) | | | [12] |
| Physarum | 0.45 (3–10) | | | |

Numbers in parentheses show the number of methylene groups examined.

monolayer lipid membrane with unit slope [23], and acceleration power of hemolysis in blood cell with slope 2 [8]. These facts suggest that action of odorants to chemoreceptive membranes has a common mechanism irrespective of species of organisms of organs.

*Comparison of Hydrophobicities in Various Chemoreceptive Membranes*

Empirical relationship given by Eq.(1) holds approximately for olfactory thresholds of human [9], rat [16] and insect [10, 11] against *n*-alcohols and *n*-aldehydes. Table 1 summarizes the values of $\Delta\mu_{CH_2}^0$ ($=\mu_{CH_2}^{0s}-\mu_{CH_2}^{0m}$) for various chemoreceptive membranes which are calculated by use of Eq.(4). Values of $\Delta\mu_{CH_2}^0$ are about 0.7–0.9 kcal/mole for *n*-alcohols and *n*-aldehydes in various chemoreceptive membranes except *Physarum*, which is about 1/2 that of olfactory organs of higher vertebrates. It is interesting to note that the difference in $\Delta\mu_{CH_2}^0$ values tabulated in Table 1 for different organisms is closely correlated with the difference in slopes in log $T$ *vs.* log $C_{th}$ plots in Fig. 9. Thus the ratios of $\Delta\mu_{CH_2}^0$ values for *Tetrahymena, Physarum* and *Nitella* against that of human give 1.3, 2.1 and 1.1, respectively. These values are compared to the slopes obtained from Fig. 9 and similar plot for *Nitella* [31], i.e., about 1.5, 2 and 1, for corresponding organisms. This fact also indicates that reception of odorants may be interpreted as the partition equilibrium of odorants between bulk solution and membrane phase.

Values of $\Delta\mu_{CH_2}^0$ for fatty acids are small as compared with those of alcohols and aldehydes. This fact cannot be explained as the difference in oil-water partition, because almost the same values of partition coefficients are obtained for these three homologous compounds [9, 16]. There-

fore, interaction of polar groups at the interface of a chemoreceptive membrane may be responsible for the difference in different homologues.

Hersh summarized values of $\Delta\mu^0_{CH_2}$ determined from minimum quantity of narcotics required to cause anesthetic action, and compared them with those determined from solubility of homologous compounds [16]. Values obtained by Hersh were between 0.7 and 0.9 kcal/mole, which were almost the same as those listed in Table 1. This fact indicates that the hydrophobic character of biological membranes is an important factor in various phenomena such as olfactory reception, narcotics, chemoreception, hemolysis, etc.

### *Sensitivity of Biological Membrane in Chemoreceptions*

Eq.(5) shows how much odorant is necessary to bring about chemotactic response or depolarization of membrane potential. The smaller the value of parameter $B$, the less $C_i^m$. High sensitivity of olfactory reception is, therefore, formally said to have a low value of $B$ in olfactory epithelium. Study of the sensitivity of chemoreception is ascribed to determination of factors which govern or alter the parameter $B$. In the protoplasmic droplets isolated from *Nitella*, it was shown that the threshold for an odorant was lowered by a factor of $10^{-2}$ with a tenfold decrease in $Ca^{2+}$ concentration in medium [32]. This fact indicated that membrane instability, or lability to change its structure, was one of the main factors governing sensitivity of the receptor membrane. The probability factor is also important in determining the threshold in olfaction, as considered by Davies and Taylor [9].

Preliminary experiments on *Tetrahymena* showed that response to lower alcohols became sensitive by more than a factor of 10 under the presence of 1-octanol. This cannot be understood if one assumes that two chemicals bind competitively on a receptor site, but rather reflects that the membrane becomes sensitive to the second chemical by the action of the first reagent. Full account of this will be described elsewhere.

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