THE LIMITING MECHANISM IN TARSAL CHEMORECEPTION*

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INTRODUCTION

The comparative effectiveness of organic compounds of homologous series in producing some given physiological phenomenon has been investigated in a wide variety of living systems. In the majority of cases there is a logarithmic increase in effectiveness, *i.e.* a decrease in effective molar concentration, as the carbon chain increases in length. It is of interest, therefore, that studies of the relative effectiveness of homologous compounds in stimulating certain chemoreceptors should reveal a modification of this generalization.

For all series of homologous aliphatic compounds investigated thus far as stimuli for the tarsal chemoreceptors of the blowfly *Phormia regina* Meigen it has been found that members of the series, when applied in aqueous solution, are indeed rejected at logarithmically decreasing concentrations as the carbon chain increases in length (4, 8, 9). In so far as logarithmic decrease in concentration is concerned these data are typical of those derived from comparable studies of such diverse phenomena as inhibition of luminescence in bacteria, narcosis of tadpoles, hemolysis of erythrocytes, etc. With tarsal thresholds, however, there is a sharp break in the curve which describes this relationship; *i.e.*, the rate of increase in stimulating effect with increasing chain length is less among the lower than among the higher members of each series. Similar results have recently been reported by Hodgson (14) for stimulation of the aquatic beetle *Laccophilus maculosus* Germ. by alcohols. Human taste thresholds, on the other hand, do not conform to this pattern (7, 16, 17).

This relationship appears to have no counterpart in any of the tabulated values for the chemical or physical properties. It has been noted (4) that the inflections in the curves for different series occur at increasing chain lengths in passing from the less to the more water-soluble compounds. In addition, the break in each series occurs consistently near that point which marks the division between those members which are miscible in water in all proportions and those with finite solubilities in water. The stimulating effectiveness of the

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latter members was shown to be inversely proportional to their molar solubility in water (10, *cf.* also 11). These facts prompted Chadwick and Dethier (4) to postulate a two phase system for the limiting mechanism in contact chemoreception in the blowfly. According to this hypothesis the smaller molecules gain access to the receptors in part through an aqueous phase while the larger aliphatic molecules penetrate through or accumulate in a lipoid phase.

It is apparent from the generally good correlation between stimulating effects and solubility characteristics that empirical threshold concentrations are greatly dependent upon the solvent, in this case water, in which the stimulating compounds are applied. Because of the unique solvent properties of water it is undesirable to express effective quantities as aqueous concentrations when investigating limiting mechanisms in comparative studies of this sort (*cf.* 3, 11). Ideally, comparisons should be made in terms of thermodynamic activities. For the majority of compounds, however, the active concentrations cannot be ascertained because values for activity coefficients and other thermodynamic functions have not been determined. The problem of determining to what extent the solvent does obscure the relationship between stimulating molecules and the receptor may be approached somewhat indirectly by investigating the effect upon threshold of substituting different solvents for water in the solutions which are applied to the tarsi. It is then possible to supplement the information thus obtained with such activity data as are available in an attempt to arrive at some clearer understanding of the process of chemoreception. For this study two solvents, mineral oil and ethylene glycol, were selected as diluents for the primary alcohols. The results together with a thermodynamic analysis of the data for a few aqueous solutions are reported herein.

Methods and Results

The principal technique used in the past to study the stimulating effectiveness of organic compounds on tarsal chemoreceptors has depended upon the fact that threshold quantities of these compounds in an aqueous solution of sugar inhibit a proboscis response to sugar (8). This inhibition occurs in the central nervous system and not at the receptor level (6); therefore, the action of the compounds on the receptors is truly one of stimulation. In order to determine what influence the solvent exerts on the system the basic technique had to be modified somewhat to permit the use of non-aqueous solvents. This was done successfully with glycol as described below. With solvents such as mineral oil, however, it is technically impossible to adapt the experimental procedure so that stimulation of the tarsi can be measured. Much useful information of another sort can be acquired by employing mineral oil solutions of alcohols as inhibitors of the sugar response at the peripheral level. This has been done. Finally the entire picture can be fitted together by comparison with another chemosensitive system, the ovipositor of *Gryllus assimilis* Fab.,

which is amenable to more direct experimental attack. The procedures which were followed in each case are described below with the results obtained.

Mineral Oil.—The selection of mineral oil as a solvent was dictated by its inertness as far as the chemoreceptors are concerned. Other organic solvents which might have been chosen are in themselves either powerful stimulants or narcotics. The particular oil employed was McKesson and Robbins' liquid petrolatum U.S.P., an oil which gave a peroxide test of 25 minutes (12). Exposure of the tarsi to this oil does not prevent a subsequent normal proboscis response to aqueous sucrose solutions.

Since it is impossible to dissolve sugar in mineral oil and determine what added concentration of alcohol would prevent a response to sugar when the two compounds are acting simultaneously, it was decided to expose the tarsi first to alcohol in oil, then to an aqueous solution of sucrose, and to observe the time required for recovery of the sucrose response. This is a very different criterion of response than used heretofore and presumably measures a narcotic activity rather than a stimulating activity. This point will be discussed in more detail later.

The experiment consisted of placing the tarsi in a mineral oil solution of alcohol of known molarity for 15 seconds and then in an aqueous solution of 0.1 M sucrose for 15 seconds. Twenty flies were exposed to each concentration and the number noted which responded to sugar within 15 seconds after removal from the oil. The test was run for a series of doubling alcohol concentrations, and a different sample of flies was exposed at each concentration. The greater the concentration of alcohol, the fewer the number of flies responding to the sugar within the allotted time. A plot of the per cent, in probability units, responding after each concentration against the logarithm of the concentration gives a straight line. Median values obtained from an analysis of these data after the method of Bliss (1) and of Miller and Tainter (15) are given in Table I.

A variant of this method was run on one alcohol (1-hexanol) as a check. In this experiment the flies were exposed to oil solutions of the alcohol as before, but instead of allowing 15 seconds for recovery, the tarsi were left in sugar until recovery finally occurred. The time was then noted. The mean time necessary for recovery of a given sample of flies did not differ significantly from one alcohol concentration to the next. However, when the number of flies recovering after 15 seconds or less was tabulated for each concentration, and plotted and analyzed as before, a median value was obtained which was not significantly different from that obtained by the first method.

An examination of the data shows that the alcohols from butanol to hexanol inclusive are effective at approximately equal concentrations. Heptanol and 2-ethylhexanol are equally effective at a lower concentration than the preceding members of the series. As pointed out before, however, the effect measured

TABLE I

The Comparative Stimulating Effectiveness of the Primary Alcohols in Different Solvents

Compound and solvent	Log molar concentration stimulating 50 per cent ± 2.575 S.E.	$a \pm$ S. E.*	$b \pm$ S. E.*	$\bar{x}$*	No. of insects tested
Tarsi of *Phormia*					
Ethylene glycol in water.	0.735 ± 0.139	4.833 ± 0.116	2.175 ± 0.280	0.658	180
Fructose in glycol	−0.848 ± 0.170	5.280 ± 0.50	2.364 ± 0.414	−0.730	100
Alcohols + 1 M fructose in glycol					
Methanol	0.934 ± 0.116	5.034 ± 0.125	2.782 ± 0.319	0.946	125
Ethanol	0.459 ± 0.139	5.011 ± 0.150	2.759 ± 0.412	0.464	100
Propanol	−0.124 ± 0.139	4.964 ± 0.144	2.608 ± 0.354	−0.138	120
Butanol	−0.104 ± 0.139	5.202 ± 0.160	3.014 ± 0.482	−0.037	100
Pentanol	−0.604 ± 0.137	5.132 ± 0.164	3.092 ± 0.500	−0.562	100
Hexanol	−1.163 ± 0.147	4.861 ± 0.153	2.700 ± 0.431	−1.214	100
Heptanol	−1.287 ± 0.108	5.003 ± 0.194	4.560 ± 0.807	−1.286	100
Octanol	−1.354 ± 0.108	5.109 ± 0.147	3.422 ± 0.495	−1.323	100
Alcohols in mineral oil (peripheral inhibition)					
Propanol	Saturated solution ineffective				50
Butanol	−0.142 ± 0.220	4.730 ± 0.151	1.698 ± 0.445	−0.326	100
Pentanol	−0.261 ± 0.203	5.063 ± 0.130	1.128 ± 0.242	−0.301	100
Hexanol	−0.338 ± 0.458	5.096 ± 0.259	0.886 ± 0.322	−0.530	100
Heptanol	−0.971 ± 0.186	5.349 ± 0.155	2.390 ± 0.436	−0.826	100
2-Ethylhexanol	−0.992 ± 0.398	5.269 ± 0.128	0.857 ± 0.154	−0.717	100
Alcohols‡ in water + 0.1 M sucrose					
Methanol	0.782 ± 0.205	4.928 ± 0.223	2.809 ± 0.662	0.757	125
Ethanol	0.377 ± 0.152	5.179 ± 0.248	4.304 ± 1.254	0.418	120
Propanol	0.077 ± 0.048	5.064 ± 0.170	9.076 ± 1.799	0.084	88
Butanol	−0.323 ± 0.066	5.363 ± 0.167	7.059 ± 1.348	−0.212	146
Pentanol	−1.122 ± 0.066	4.919 ± 0.191	7.507 ± 1.272	−1.132	105
Hexanol	−2.211 ± 0.136	4.991 ± 0.145	2.742 ± 0.491	−2.213	100
Heptanol	−2.935 ± 0.189	4.848 ± 0.142	1.968 ± 0.370	−3.012	100
Octanol	−3.940 ± 0.161	4.832 ± 0.150	2.445 ± 0.414	−4.008	100
Ovipositor of *Gryllus*§					
Alcohols in mineral oil					
Propanol	−0.886 by graphic interpolation				25
Butanol	−0.770 " " "				25
Pentanol	−0.854 " " "				25
Hexanol	−0.886 " " "				25
Heptanol	−1.367 ± 0.186				80
2-Ethylhexanol	−1.268 ± 0.132				150
Alcohols in water					
Methanol	1.041 by graphic interpolation				30
Ethanol	0.568 ± 0.126				112
Propanol	0.041 ± 0.129				140
Butanol	−0.959 ± 0.152				80
Pentanol	−1.481 ± 0.108				80
Hexanol	—				
Heptanol	−2.770 by graphic interpolation				30
Octanol	—				

S. E. = standard error

* The 3d, 4th, and 5th columns of the table give the calculated values for a, b, and $\bar{x}$ in the equation $Y = a + b(X - \bar{x})$, which is the regression of the per cent of the insects stimulated, Y, expressed as probits, on log concentration, X.

‡ Data are taken from Dethier and Chadwick (9).

§ Calculated according to the method of Miller and Tainter (15).

here differs from the rejection threshold usually measured so that a comparison of the two sets of data is not valid in the absence of additional information. In an attempt to ascertain whether the relative narcotizing effect in oil reflected in any way the order of stimulating effectiveness, a comparison was made with another system, the ovipositor of the cricket *Gryllus*, in which the stimulating effectiveness could be measured directly.

The ovipositor of *Gryllus*, like those of certain hymenopterous parasites, is sensitive to stimulation by various electrolytes and organic compounds (5). When the ovipositor is placed in a threshold concentration of any of these compounds, a response is elicited which consists of a rapid vibration of a tetanic nature followed by movements of greater magnitude if stimulation is continued or if the initial stimulus is considerably above threshold. The advantage attendant upon use of this preparation lies principally in the fact that direct stimulation is measured rather than prevention of a response to sugar. Since sugar is not required, a variety of inert solvents may be employed.

A series of tests of the stimulating effectiveness of aqueous solutions of the primary alcohols on the ovipositor showed that this organ responds in essentially the same manner as the tarsi of *Phormia*. Median rejection thresholds for the first eight alcohols are plotted in Fig. 1. The alcohol curve for the tarsi of *Phormia* is included for comparison (Fig. 1). Rejection thresholds for mineral oil solutions of propanol through 2-ethylhexanol were determined in exactly the same manner. These results are given in Table I and also plotted in Fig. 1. The agreement between these data and those obtained for narcosis of tarsi is striking. Here also the three to six carbon alcohols act at approximately equal concentrations and the eight carbon one at a lower concentration.

Glycol Solutions.—Ethylene glycol is ineffective at all concentrations in eliciting a proboscis response from *Phormia*. That it does have a stimulating effect is shown by the fact that threshold concentrations will prevent thirsty flies from accepting water containing it. The median rejection threshold obtained in this manner is 5.432 M. In order to use ethylene glycol as a solvent in tests with alcohols, therefore, it is necessary to add enough sugar to overcome its inherent repellency. Since sucrose is not sufficiently soluble to give the required concentration, glycol solutions of fructose have been used. All glycol solutions were 1 molar with respect to fructose. This high concentration of fructose was chosen to induce a high per cent of response from the flies. The median acceptance threshold for fructose in glycol is 0.1142 M. The same factor for fructose in water is 0.0058 M.

The rejection thresholds for the first eight primary alcohols in glycol solution are given in Table I.

DISCUSSION

Since the values representing the physiological action of the primary alcohols in different kinds of solutions have been obtained under dissimilar sets of

conditions, some transformation is necessary before comparisons can be drawn. Of the three sets of data obtained for tarsal receptors, the thresholds measured in aqueous solution represent the concentrations necessary to prevent a proboscis response to 0.1 M sucrose; those in glycol, the concentrations required to prevent a response to 1 M fructose; those in mineral oil, the concentrations which narcotize or otherwise prevent the sugar receptors from functioning for a given period of time. The first two sets of data can be reduced to a common

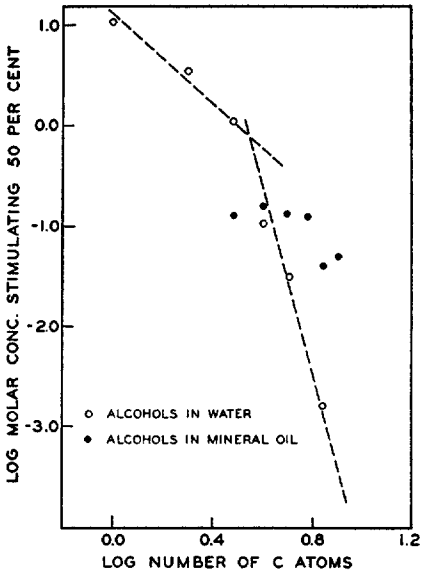

FIG. 1

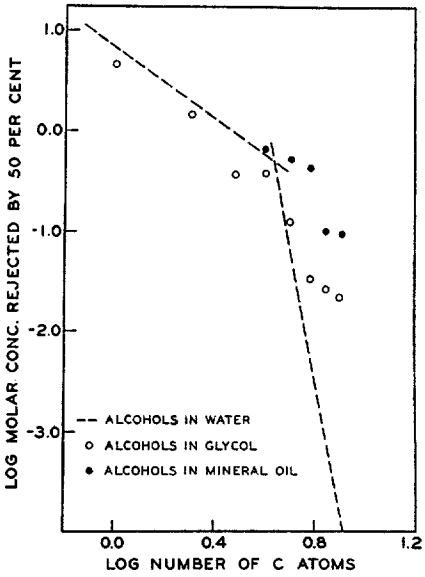

FIG. 2

FIG. 1. Stimulating effectiveness of primary alcohols applied to the ovipositor of *Gryllus* as aqueous and as mineral oil solutions. The broken line represents the curve for the tarsi of *Phormia*.

FIG. 2. Rejection thresholds of aqueous and glycol solutions of primary alcohols by *Phormia*.

base by correcting for the difference in stimulating effect of sucrose and fructose and for the difference in concentrations employed in each case. The median acceptance threshold for fructose in water is not significantly different from that of sucrose. For fructose the value is 0.0058 ± 0.0007 M; for sucrose, 0.0098 ± 0.0032 M (13). The slopes of the lines of regression of per cent flies accepting sucrose and fructose respectively are 1.045 ± 0.168 and 3.252 ± 0.428. Hence no appreciable error is introduced if the difference in stimulating effectiveness of the two sugars is ignored. A correction for concentration can be made by extrapolation of the line which describes the relation between the

concentration at which glycol is rejected in different sugar concentrations and the concentration of the sugar (Dethier, in press). The corrected values for the glycol solutions are plotted in Fig. 2.

The values for mineral oil cannot be transformed because an entirely different physiological phenomenon is being measured. It might be argued, however, by analogy with ovipositor data, that the narcotic thresholds of the alcohols in mineral oil bear the same relation to one another as do their stimulating thresholds. The pattern of the data is strikingly similar for both insects.

It is clear from these experiments that the observed comparative stimulating effectiveness of the alcohols assumes a different aspect with each different solvent. In oil the range of thresholds extends over less than one log unit of concentration as compared with the corresponding thresholds in water which extend over four log units. In glycol the thresholds extend over two and one half log units only and do not exhibit the sharp break characteristic of the water line.

Considering first the relation of oil thresholds to water thresholds one observes (Figs. 1 and 2) that in general the lower alcohols are equally or more effective, on a molar basis, in mineral oil than in water, while the higher members are considerably more effective in aqueous solution. If, as the original hypothesis envisions, the lower members stimulate *via* an aqueous phase and the higher members *via* a lipoid phase, a qualitative explanation which would fit the facts can be made. The lower members by virtue of hydrogen bonding to water and infinite water solubility would tend to remain in aqueous phase. Consequently for effective stimulation lower concentrations would be required at an aqueous phase receptor than at a lipoid one. Actually, as Fig. 1 illustrates, these alcohols are more effective than would have been anticipated were a single phase system involved. When they are presented in mineral oil solution, there is a tendency for them to escape readily to the receptor aqueous phase; hence, when in mineral oil still lower concentrations would be required for stimulation. With higher members of the series the reverse situation would prevail. There would be a greater tendency for the higher alcohols to escape into the receptor lipoid phase from an aqueous solution than from an oil solution; consequently, higher concentrations must be used when applied in mineral oil than in water. Glycol may be considered as representing a solvent intermediate between water and mineral oil, and indeed its influence on the stimulating effectiveness of the alcohols is intermediate between the influence of water and that of oil. As already pointed out the line describing the effectiveness of alcohols in glycol solution extends over two and one half log units as compared to the range of four log units in aqueous solution and less than one in oil.

If the limiting process of chemoreception described here depends upon an equilibrium being established between the concentration of alcohol in the ambient solvent and that in the receptor, then further indications as to the

homogeneity of the system might be revealed by a thermodynamic analysis of the data for alcohols in water. Furthermore, the calculated thermodynamic activities at threshold should compare favorably with the empirical thresholds determined in oil since there is reason to believe that the activity coefficient for each alcohol in oil is close to unity.

Aqueous molar threshold values can be converted to thermodynamic activities by multiplying the mol fraction of the alcohol at threshold by the aqueous activity coefficient at infinite dilution and 25°C. (3). When the products are plotted against the activity coefficients on logarithmic coordinates

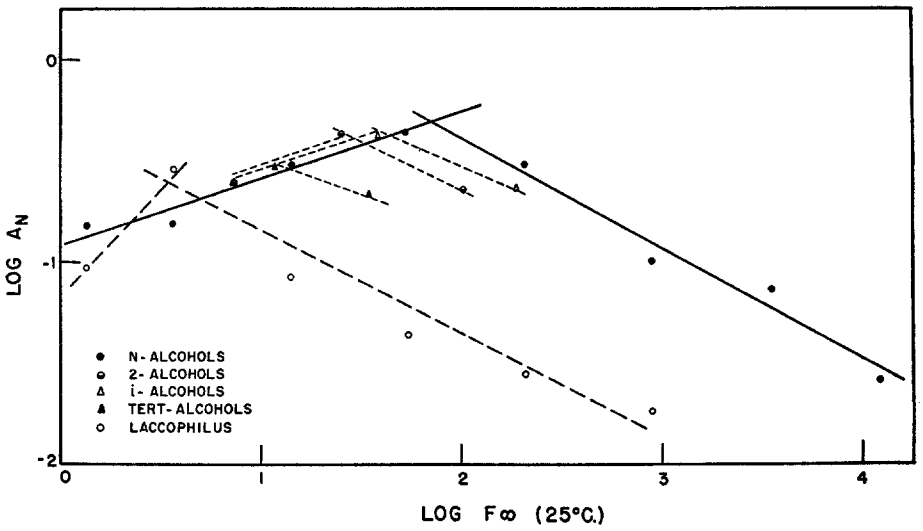

FIG. 3. Comparison of the rejection thresholds, expressed as thermodynamic activities, of the primary, secondary, and tertiary alcohols by *Phormia* and the primary alcohols by *Laccophilus*.

(Fig. 3), it is clear that the alcohols fall into two groups. The first six members roughly approximate a constant value. The seven and eight carbon members are definitely lower. The molar thresholds in oil exhibit a similar arrangement with respect to one another. On the other hand, there is an obvious tendency for the activities to increase from the one to the five carbon member and to decrease from that point to the eight carbon member. The discontinuity in the five carbon region is obvious.

It is apparent that the relation of equal physiological effect at equal thermodynamic activities is not in evidence here. Nor do the data follow a progressive increase as described for many systems by Ferguson (11) and Badger (2). Either the process of tarsal chemoreception does not depend upon the establishment of an equilibrium or else some kinetic effect is obscuring an underlying relationship which does depend upon an equilibrium. Other evidence supports

these views. For example, conversion of molar thresholds of compounds of other homologous series to activities does not bring the compounds into one homogeneous system with the alcohols. Thus the secondary and tertiary alcohols cannot be brought into line with the primary alcohols by this procedure. Nor do the ketones fall into line. It had been shown earlier (10) that for compounds with measurable solubilities in water there was an inverse relationship between stimulating effectiveness and water solubility and that threshold values for individual compounds frequently were significantly different from those which would be expected solely on the basis of correlation between threshold and solubility. It was to be expected that if uncomplicated equilibrium phenomena were involved, such deviations could be accounted for, at least partially, by conversion of threshold values to activities and that the analysis might be extended to those compounds for which values for solubility could not be obtained (*e.g.* infinitely miscible). The aforementioned analysis of the isomeric alcohols and ketones shows, however, that this expectation is not realized. While there is a tendency on the one hand for all the isomers of any one alcohol to be stimulating at equal activities, they are not all brought into a single homogeneous system, and it is still possible to resolve each set (*e.g.* the secondary alcohols) into its own series the line of which parallels that of the primary alcohols and exhibits a similar break.

Unfortunately the water, glycol, and oil data are not strictly comparable on a thermodynamic basis because the activities of alcohols in glycol are not known and the mol fraction in oil cannot be calculated. However, water/mineral oil partition coefficients have been determined experimentally and the coefficient for each alcohol compared with the ratio of the water threshold to the oil threshold. There is no obvious agreement between these values, neither for the ovipositor nor the tarsi. This lack of agreement may be construed as additional evidence that no equilibrium phenomenon is involved or that it is being obscured by kinetic effects.

Since the deviations in the action of alcohols on tarsal and ovipositor systems are peculiar to those systems and are not to be ascribed solely to intrinsic characteristics of the compounds themselves, it appears that the explanation is to be sought in unique features of the cells or tissues involved. Further evidence in support of this idea may be adduced from a comparison of the action of homologous compounds on other chemoreceptive systems. Threshold data are available for alcohols acting on human beings (16), alcohols on the beetle *Laccophilus* (14), glycols on *Phormia* (9), and glycols on man (7). In each series of data pertaining to insects the threshold/chain length relationship exhibits a discontinuity. The location of the break is not identical for each species. It lies between butanol and pentanol for *Phormia* and between propanol and butanol for *Gryllus* and *Laccophilus*. In both series of human thresholds there is no pronounced break in the curve (*cf.* 7).

The underlying reasons for the relationships displayed here are not known

with certainty, and several alternative interpretations are conceivable. The evidence offered, however, strongly supports the hypothesis that the limiting mechanism in tarsal chemoreception involves a two phase system. The identity of the receptors involved, the nature of their innervation, and other details of structure as well as the actual process of stimulation await further investigation.

SUMMARY

Rejection thresholds of eight primary alcohols applied to the tarsal chemoreceptors of the blowfly *Phormia regina* Meigen and the ovipositor of *Gryllus assimilis* Fab. have been determined. Three different solvents for the alcohols have been used: water, ethylene glycol, and mineral oil. The comparative stimulating effectiveness of the alcohols assumes a different aspect with each different solvent. In oil the range of thresholds from methanol to octanol extends over less than one log unit as compared with the corresponding thresholds in water which extend over four log units. In glycol the thresholds extend over two and one half log units only. When water is employed as a solvent, the line which describes the relationship between threshold concentration and chain length of the compound exhibits a sharp break at or near butanol. No such discontinuity is evident when glycol or oil is employed as solvent. This is offered as evidence supporting the hypothesis that the limiting mechanism in tarsal chemoreception involves a two phase system whereby highly water-soluble compounds gain access to the receptor through an aqueous phase and the larger lipoid-soluble molecules chiefly through a lipoid phase. Additional facts which support this idea are gained from data which show that the inflection in the curve occurs at different points with different species of insects and is conspicuously absent in the case of man.

When thresholds in aqueous solutions are converted from molar concentrations to activities, it is clear that the relation of equal physiological effect at equal thermodynamic activities does not apply here. The lower members of the series stimulate at progressively increasing activities up to pentanol and then at progressively decreasing activities. Furthermore, the ratio of water threshold to oil threshold exhibits no obvious agreement with the water/oil partition coefficients determined experimentally. These results indicate either that the limiting process of chemoreception in these insects does not depend upon the establishment of an equilibrium or that some kinetic effect is obscuring an underlying relationship which does so depend.

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