PHARMACOLOGICAL REVIEWS
Vol. 22, No. 1
Printed in U.S.A.

THE MOLECULAR PARAMETERS OF OLFACTORY RESPONSE

M. G. J. BEETS

International Flavors & Fragrances (Europe), Hilversum, Holland

TABLE OF CONTENTS

I. INTRODUCTION

The olfactory system in vertebrates can be described in very simple terms as consisting of the olfactory epithelium in which the information originates, the olfactory nerve in which it is transported, and the olfactory bulb in which it is processed for delivery to the higher centers. In the brain the olfactory code message is translated into an odor sensation and into patterns of recognition and association which, in man, may be converted into a verbal expression of the sensation.

The olfactory epithelium is a densely packed structure of receptor cells and supporting cells. Its surface consists of the peripheral membranes of both cell types, each of which carries hair-like filaments which are embedded in the thin mucous layer covering the epithelium. The supporting cells probably play some role in the mechanism of signal formation (65, 92) but they contain no receptor sites capable of interaction with odorant molecules (88). We may safely assume that olfactory stimulation and the formation of olfactory signals take place on and in the receptor cell. Although conclusive evidence is still lacking, the membrane of the filaments of the receptor cells in vertebrates, the cilia, is generally thought to be the scene of interaction with odorant molecules and to contain the receptor sites (71).

The olfactory nerve consists of nerve fibers, each of which connects a single receptor cell, without branching, with a glomerule in the olfactory bulb. The latter may be considered as the information processing and output unit of the system. Its most characteristic elements are the glomerules and the mitral cells. We know that single nerve fibers enter a glomerule from many different directions. Of the 50 million receptor cells in the rabbit, 25,000 are directly connected with a single glomerule, of which there are about 1900. The strong convergence taking place in the glomerules suggests that their major function consists of information processing, including classification and elimination of nonessential components. The resulting information pattern is redistributed over a second layer of processing units, the mitral cells, of which there are 48,000 in the rabbit.

This very brief outline of the olfactory system suffices for the purpose of this paper. Extensive reviews on this subject have been published recently by Gasser (49), de Lorenzo (64), Reese (83), and Farbman (48).

The process of olfaction is initiated by the interaction of odorant molecules with the mucosa containing the olfactory nerve endings. Although for most types of odorants, especially those with low molecular weight, trigeminal and vomeronasal nerve endings also play a part in the formation of the olfactory code message, we may assume that the impulses generated in the olfactory membrane carry the major part of the total information and, consequently, are largely responsible for the discriminative power of the olfactory system.

In the early stages of research on olfaction the interaction between the odorant molecule and the olfactory mucosa served as the playing ground for many highly imaginative theorists. Since then, the increasing application of experimental techniques to the problems of olfaction has contributed much to our understanding. Theories based on tele-activation of the receptors have now been abandoned (78) and others that assumed a chemical or enzymatic conversion of the odorant molecule in the interaction process are no longer tenable in view of the endless diversity of odorant structures. This does not mean that the receptor site may not have an enzymatic function which is triggered by absorption of a suitably shaped odorant molecule. A mechanism of this type, which does not involve a chemical conversion of the odorant molecule, has recently been proposed by Martin (66).

At the present stage of our knowledge, it is generally accepted that the interaction between odorant molecules and receptor components takes place in the framework of an absorption-desorption process from which the odorant molecules emerge with unchanged structure.

In terrestrial animals the zone of interaction is an interface between a mucous layer and the receptor membrane. Odorous molecules are continuously supplied to and removed from the mucus by a current of air. The mucus, which because of its viscous, colloidal character, is probably an ideal medium for the absorption and transport of molecules of highly divergent structures, is, in its turn, responsible for a continuous supply to and removal from the receptor surface of odorant molecules. This also holds good for the microlayer of mucus which is in immediate contact with the receptor surface. If this layer, which may be called the carrier phase, is defined as being sufficiently thin, it may be considered to be stagnant and to represent a steady state with respect to odorant concentration during the major part of the inspiration. This concentration determines the nonintrinsic term of the odor intensity.

The zone of interaction which can now be defined more accurately as the combination of the carrier phase and the receptor surface, is the site at which the olfactory process starts. An odorant molecule is absorbed from the carrier phase onto the receptor surface. After a certain time of residence, the length of which depends on the affinity between the molecule and the molecular receptor site with which it combines, it is released into the carrier phase, from which it may be either absorbed again or removed by more remote layers of mucus and, ultimately, by air.

During the period of absorption, olfactory interaction takes place. All experimental information available, some characteristic examples of which will be discussed in this paper (for a more extensive account, see 22), strongly indicates that molecular shape rather than chemical nature is the decisive criterion for the quality of the odor sensation and its precursors (7, 18, 81, 91).

Accepting reversible absorption across an interface as a safe starting point for the processes involved in olfactory interaction, we may now ask what we can expect on the basis of our knowledge of analogous conventional absorption processes in which large structurally homogeneous populations of molecules are involved.

Such processes are statistical phenomena. Although all molecules of a given compound have identical structures they will be absorbed at the interface in a pattern of orientations, the degree of randomness of which is determined by the nature of the polar groups in the absorbed molecules and by the polarity of both phases.

A population of identical, rigid, and nonpolar molecules will enter the interface in an almost random pattern of orientations. The orientation pattern for rigid, polar molecules, possessing a sterically accessible functional group can be expected to be much more homogeneous.

As polarity or steric accessibility of the functional group decreases, the orientation pattern degenerates towards randomness. Absorption of flexible molecules at an interface is governed by variations of orientation as well as of conformation and presents a much more complex pattern.

We may now ask how far this outline of molecular behavior at a conventional interface between two layers is applicable to the absorption process involved in olfaction. In this case, the nonaqueous phase has a structure consisting of or containing sites on the polymolecular, monomolecular, or submolecular level with a roughly specific tendency to accommodate specific molecular profiles with varying efficiency in a reversible complex between molecule and receptor site.

Nonpolar molecules will probably adapt their conformation to the steric requirements of the sites to which they are presented as far as their orientation and their flexibility permit. Since only one of the partners (the olfactory receptor site) has a polar character, there is no driving force towards re-orientation, and the molecules can be expected to participate more or less efficiently in the interaction complex in the orientation in which they arrive, or to be released without forming such a complex in cases where the steric requirements of oriented profile and site are incompatible. Consequently, a population of nonpolar molecules with identical structures will be presented to the population of sites as having an almost infinite variety of shapes. Interaction in this case will result in a blurred olfactory code message which the brain is unable to identify or analyze, much as it is unable to identify a blurred photograph of a fast moving object (20). This is roughly in agreement with the weak, uncharacteristic odors of pure, saturated hydrocarbons.

Rigid, polar molecules will participate more or less efficiently in the interaction complex mainly in a single orientation dictated by their own polarity and by that of the sites. Perhaps the most characteristic example of this type is presented by the musks (18).

Finally, flexible polar molecules have an extra degree of freedom in the adaptability of their conformation to the steric requirements of the sites, which differ for different types of sites. Such molecules can participate in interaction complexes with as many different profiles as there are site-types available for interaction.

Since there is an obvious relationship between molecular structure and molecular behavior in absorption processes, including the absorption process involved in olfactory interaction, we may attempt to answer the question how this behavior can be expected to influence the olfactory code message.

The interaction of each individual molecule with a molecular receptor component results in the formation of an elementary bit of information. The information pattern initiated on the membrane (the primary information pattern) is an extremely complex assembly of such elementary bits. It is subjected to a number of processing steps in the receptor cell body and in the glomerules and the mitral cells of the olfactory bulb. Although experimental evidence for the meaning of these steps is still lacking, it seems attractive to assume that they result in a classification of the initial information and in elimination of nonessential complexity. Their terminal product is the olfactory code message which is delivered to the brain, containing all essential information originated in the interaction.

The complexity of the code message is undoubtedly related to the number of different types of elementary bits in the primary information pattern. This complexity can be expected to decrease with decreasing molecular flexibility, increasing polarity, and increasing steric accessibility of the functional group.

Identifiable odor types, characterized by easy recognition and large populations of representatives, in short the types named "primary odors" by Amoore (7) can be expected to be related to primary information patterns of minimal complexity (maximal homogeneity) and consequently to occur mostly in the classes of fairly rigid, polar molecules such as musks and camphors and never in nonpolar molecules such as saturated hydrocarbons.

The orientation pattern in the absorption process of even the most rigid and polar molecules can never be completely homogeneous, *i.e.*, it can never consist of a single oriented molecular profile. Also, the receptor sites are only roughly stereospecific. A specific site can accommodate a certain oriented molecular profile with maximal efficiency but various other profiles with less efficiency. This means that even the most homogeneous interaction pattern will consist mainly of one specific and highly efficient molecular component but that it will contain always other, less efficient components occurring in lower frequencies.

The ideal "mono-osmatic" odor (21), related to a code message consisting of only one type of component, cannot exist. It is perhaps approximated in insects by stimulation of specialized receptor cells, *e.g.*, the queen substance (59). All odor sensations as well as their precursors tapped from other parts of the olfactory system are necessarily complex and consequently cannot be fully identical for two structurally related odorants. What we recognize in primary odors is related to the component occurring with maximal frequency in the primary information pattern as well as in the final code message. This component is shared by a large group of odorants. But the olfactory message is invariably blurred by components of minor importance which differ for the members of a single group of primary odorants.

Finally we should mention two consequences of the absorption character of olfactory interaction which are of practical importance for our studies on the relationship between odorant structure and olfactory response. First, the preferred conformations of flexible molecules in the olfactory interaction complex are not necessarily identical with those of free molecules or molecules absorbed at an unstructured interface, since we can expect the molecule to adapt its conformation to the steric requirements of the site to which it is presented. Since we know nothing of the latter, the practical consequence is that it seems justified to compare molecular models of compounds with similar odors in conformations with maximum profile similarity. Second, the preferred orientation of a polar molecule on a specific receptor site can be assumed to be related roughly in the same way to the direction of the dipole axis for molecules with similar odors. The degree of profile similarity for such compounds should be studied by comparing their molecular models in the same orientation with respect to the dipole axis.

We shall see in the course of this paper how far these tentative conclusions are corroborated by experiments.

The principles of absorption, molecular profile, and orientation, indicated above, are involved in any absorption phenomenon and we may safely assume them to play some part in olfactory interaction. As soon as we step beyond these principles, we enter the realm of pure speculation.

All we know about the nature of the receptor sites is that they are probably located on proteinaceous (lipoprotein) molecules or on matrices of such molecules. Davies (34) assumed that the polymolecular matrix forming the receptor membrane is punctured by the odorant molecule. Amoore (7) started from the hypothesis that the receptor membrane contains permanently available cavities of specific shape. The present author, for reasons of analogy, slightly favors a somewhat different concept, according to which a molecular receptor component or part of such a component undergoes a configurational change in order to accommodate the steric requirements of the absorbed odorant molecule. However, all three concepts are purely speculative and, particularly for the subject of this paper, the choice is arbitrary since a potential receptor site may have the same steric characteristics as a permanently available one.

Also the mechanism of olfactory interaction opens a field for speculation which we will avoid in this paper. All that really interests us here is the question whether there exists a relationship between (a) the expected orientation pattern, the size and dimensions of the preferred oriented profile and perhaps some other structural parameters of a homogeneous population of odorant molecules and (b) the characteristics of olfactory information caused by interaction of such molecules with the olfactory receptors, no matter where in the olfactory system the signals are recorded.

Since we are unable to relate the structural parameters of a single odorant to the olfactory response, our approach can only be based on comparison. We may either attempt to detect similarities in the response patterns caused by stimulation with odorants of similar structure or to correlate differences between such patterns with gradual structural changes such as those in the members of a homologous series. Since the first method is by far the most important we have selected similarity as the guiding theme for this paper.

Structural similarity, *i.e.*, in the context of this paper, similarity between oriented profiles, can be assessed roughly by comparing the morphological characteristics of molecular models. Recently Amoore *et al.* (14) have put the assessment of structural similarity on a more quantitative basis by using computer techniques, and we may hope that this will ultimately result in a quantitative expression of the degree of structure similarity.

Response similarity is a more complex problem. Our sources of information on olfactory response are either subjective (verbal expression of the odor sensation in man, behavioral response in higher animals) or objective but obtained from an organism functioning under abnormal conditions (electrophysiological recordings, behavioral response in lower animals). The disadvantages of these approaches may partly be neutralized by comparing the responses of the same organism, individual or group obtained by stimulation with two structurally related stimulants. In this way the unknown distortions of the information can be expected to be parallel and roughly equal. Statistical treatment of subjective information can largely eliminate distortion. Finally, a small number of phenomena which are beyond the consciousness of the subject enable us to obtain objective information by means of techniques which normally give subjective information.

In the following pages some important techniques will be explained and illustrated by means of a few carefully selected results. More complete reviews of the literature are, or will soon be available for the interested reader (18, 19, 22).

II. SIMILARITIES OF ODORS

Two sources of light may be observed as having closely similar colors if both emit light of similar spectral composition but also if one of them emits monochromatic light and the other a spectral composite.

Theoretically the same distinction between fundamental similarity and pseudosimilarity could exist in olfaction. The former would occur when the olfactory code messages originated by interaction of the epithelium with two odorants would have closely related informational compositions. In the case of pseudosimilarity two olfactory code messages of vastly different informational content may happen to be translated by the brain into similar odor sensations. Observations on specific anosmia and cross-adaptation to be discussed in subsequent paragraphs provide some cogent arguments for the actual existence of both types.

Since the mere statement that the odors of two chemicals are closely related does not enable us to distinguish between fundamental and pseudosimilarity, the use of verbal odor expressions for the detection of odor similarities may result in erroneous classifications and futile searches for nonexistent structural relations. Especially in cases where odor similarity is observed between chemicals with widely different structure and molecular shape, conclusions should be avoided until other techniques have been applied to confirm that the relationship is a fundamental one. We must keep this in mind when reviewing in this section some characteristic examples of odor similarity based on the verbal expressions of odor.

Odor classification based on the assessment of odor similarities may be a source of information on the mechanism of olfaction. An attempt to describe the odors of all known organic chemicals with molecular weights up to about 300 in terms of key-words would result in a limited number of key-words and a large number of odorants under each key-word. The odors of most chemicals can be described with reasonable accuracy only by a number of key-words, and although many others would occur under a single key-word, we would find that not two of these have exactly the same odor quality. This means that even odorants for which the limited semantic tools available seem to suggest a homogeneous "mono-osmatic" odor type produce more or less complex sensations derived from information patterns in which one component predominates, but in which secondary ones always occur. This agrees well with the theoretical arguments given in the preceding paragraph.

Although a frequency diagram representing the distribution of the total population of odorants over an imaginary continuum of odor types would not show any isolated peaks, it would certainly show a continuous curve with a number of peaks of varying height. It seems reasonable to assume that each of these peaks would represent a single type of response originating in a single type of receptor site and that a catalog of such peaks would contain a representation of all the fundamental components of any olfactory information pattern.

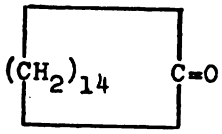
On the basis of a similar philosophy, Amoore (7, 8) has recorded the key-words found in a large collection of data from the literature. The number of fundamental odors that can be discovered by this method is limited to those which can be characterized by key-words, but the principle is promising as a serious test of our theoretical concepts.

Amoore's semantic analysis of the literature resulted in the discovery of 7 primary odor classes, characterized by the key-words: camphoraceous, musky, floral, pepperminty, ethereal, pungent and putrid. Statistical tests (11, 15) in which the odor similarity estimated by a panel and the profile similarity of each of 107 odorants were correlated with those of representatives of each of 5 primary classes, yielded satisfactory degrees of correlation. The pungent and putrid classes, which are believed by Amoore to depend on the nature of the functional group rather than on the profile, were omitted.

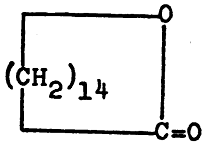
Later, on the basis of his work on specific anosmia, Amoore (13) postulated the existence of a much larger number of primary odors.

Accepting the theorem that, because of the complexity of the olfactory information patterns from which the odor sensations are derived, no two odorant can have exactly the same odor, we can approach odor similarity only by studying how far we can change a structural detail of an odorant molecule without changing the odor beyond recognition or, in more theoretical terms, without pushing the major component of the olfactory information pattern into a secondary position.

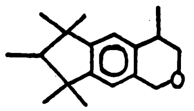
Most of the examples in the following discussion of odor similarity have been taken from the musk class. The reason is that we know far more representatives of this odor type than of any other. Apart from continued commercial interest this is undoubtedly due to the rather simple structural requirements for the musk odor, *i.e.*, a fairly rigid, closely packed structure with a molecular weight roughly between 210 and 310 and a sterically well accessible group. Theimer and Davies (90) have refined the profile criterion for musks further by defining the ratio between two dimensions of the oriented profile and the cross-sectional



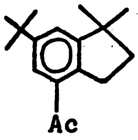
I



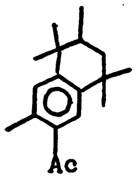
II



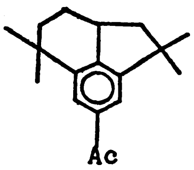
III



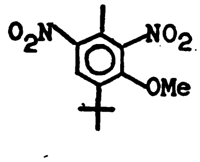
IV



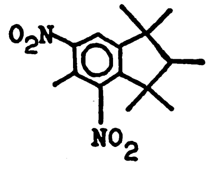
V



VI



VII



VIII

area. Extensive reviews on musk structure have been published by Beets (18, 20) and by Wood (94). The structures I–VIII indicate the wide structural scope of the musk odor. In subsequent paragraphs we shall see that the fundamental homogeneity of this superclass is highly questionable. For this reason the examples selected in this paragraph belong to subclasses of which the homogeneity is much more certain.

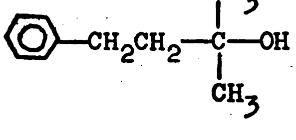
The molecular weight of odorant molecules in general has no lower limit. Chemicals with small molecules such as formaldehyde elicit odor sensations with strong trigeminal contributions. The upper limit lies just beyond 300. The molecular weight limits for specific classes of odorants seems to be characteristic for each class and even for each subclass.

In the introduction we have stressed the importance of the oriented profile for the odor type. The most direct demonstration of this can be given by replacing certain parts of the profile in such a way that shape, size, and orientation remain virtually unchanged. The materials available for such a remoulding of the profile are rare since we can only replace carbon and hydrogen by other atoms and this usually introduces a factor influencing polarity and orientation pattern.

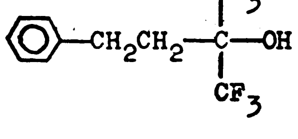
Obvious examples are the replacement of hydrogen by fluorine or deuterium and of methyl by bromine. Theimer (89) found the odors of the well known odorant dimethyl-phenylethylcarbinol (IX) to be similar to that of the hexafluoro analog X. Also several examples are available demonstrating that the musk odor is often retained by replacement of methyl by bromine but also by other groups such as methoxy, chlorine, or cyano (94).

The importance of orientation as a factor in molecular shape can be demonstrated by elimination of the functional group or by decreasing its steric accessibility without changing seriously the morphology of the profile. In both cases the orientation pattern can be expected to degenerate towards randomness, resulting in complete loss of the odor character. An example of the elimination is the replacement of the acetyl group in the musk IV by ethyl, leading to an odorless hydrocarbon. Similar changes in smaller molecules yield hydrocarbons which are not necessarily odorless but which have the atypical "blurred" hydrocarbon odor.

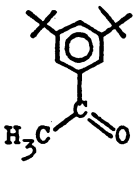
The orientation of molecules at an interface between two phases of different polarity is associated with the solvation tendency of their functional groups, and consequently the steric accessibility of the latter is a decisive factor for the homogeneity of the orientation pattern. If this also holds good for the situation at the receptor membrane, we may expect also in this case a loss of odor character. That this does actually occur is demonstrated by the examples XI–XX.



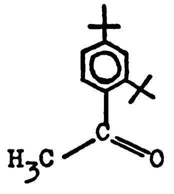
IX



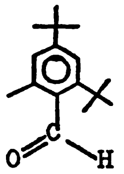
X



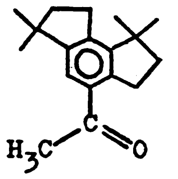
XI



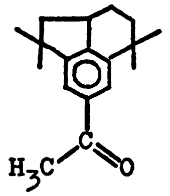
XII



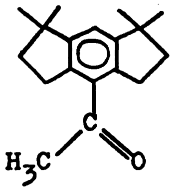
XIII



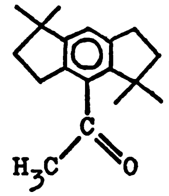
XIV



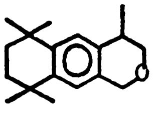
XV



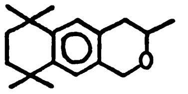
XVI



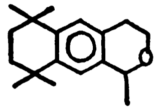
XVII



XVIII



XIX

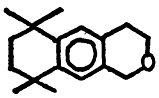


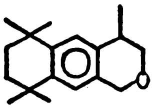
XX

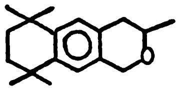
The isomer XII of the moderately strong musk XI, is odorless. Its carbonyl group is sterically hindered by the neighbouring tertiary butyl group and does not form an oxime at room temperature. This is not caused by the different position of the tertiary butyl group since the isomer XIII, in which they have the same position but the accessibility of the carbonyl group is better (slow oximation), is a fairly weak musk. The ketones XIV and XV, with freely accessible polar groups, are both strong to very strong musks, but the isomers XVI and XVII, in which the carbonyl groups are increasingly hindered, are fairly weak and very weak musks. Finally, shifting the methyl group in the very strong musk XVIII to either one of the positions vicinal to the oxygen atom (XIX, XX) destroys the odor.

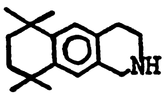
Replacement of the polar group of an odorant molecule by another in the same structural position may be expected to influence the odor only insofar as it changes the statistical composition of the orientation pattern and the absorption affinity at the membrane or the morphology of the oriented profile. This means that many such replacements may result in a reasonable degree of retention of the odor character. This is confirmed by a large number of examples of which a few are mentioned here. Classical examples are the retention of the bitter-almond odor in the series benzaldehyde, nitrobenzene and benzonitrile. In the same way the characteristic odor of piperonal may be retained by replacement of the aldehyde group by an azide function. A more interesting example is found in the comparison of the musk series XXI–XXIII and XXIV–XXVI. XXI is a musk of medium intensity and its 4-methyl-homolog XXII is a very strong musk whereas the isomer XXIII with the methyl group in the position vicinal to the functional group is odorless. The same trend, on a lower intensity level, is observed in the series XXIV–XXVI in which the oxygen has been replaced by a secondary amino function.

Interesting effects are produced by the presence of a second profile group or of a second functional group in an odorant structure. The first case can be expressed schematically by XXVII, in which F represents the bivalent polar group and A and B the profile parts. As long as A is small (H or CH_3) the oriented profile is almost entirely defined by the dimensions of B, but as A grows in size it starts contributing to the profile of the predominating component of the orientation pattern and we may expect changes in odor character. Examples of this effect have been described by von Braun and Kröper (29) for a series of undecanones (XXVIII) and by Beets (21) for a series of keto musks (XXIX). In the former series, undecanone-2 (n = 0) has a strong odor of rue. As n in-

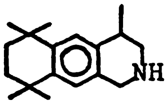

XXI


XXII

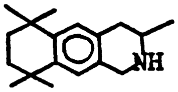

XXIII



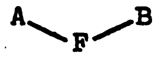
XXIV



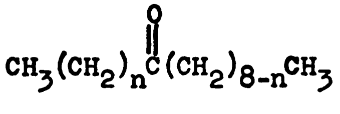
XXV



XXVI

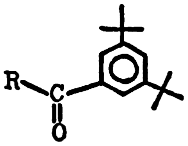


XXVII

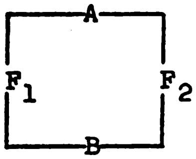


$CH_3(CH_2)_nC(=O)(CH_2)_{8-n}CH_3$

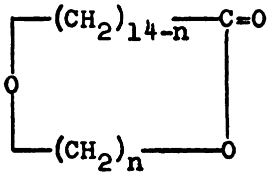
XXVIII



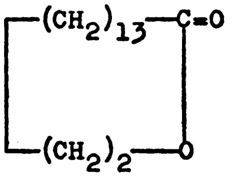
XXIX



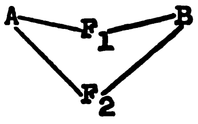
XXX



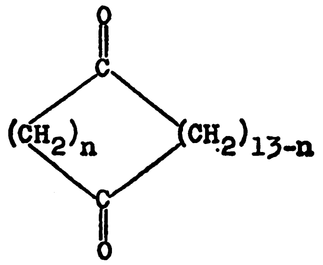
XXXI



XXXII



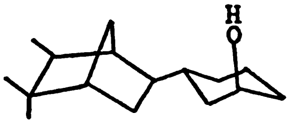
XXXIII



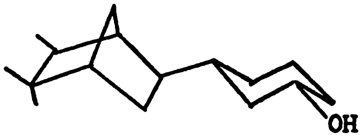
XXXIV

creases from 0 to 4 the rue odor weakens and is replaced by a fruity, amylacetate-like odor of increasing intensity. When R in XXIX is hydrogen, we observe a musk of medium intensity, blended with an anisic note. The latter disappears for R = CH_3 and is replaced, in the ketones with ethyl and isopropyl groups, by a sweet note of which the intensity increases while the musk intensity decreases.

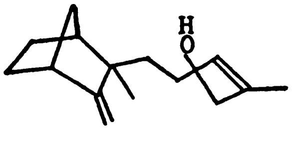
The effect of a second functional group may be expected to depend on the distance between the two groups and upon their nature. In a cyclic structure of type XXX, in which F_1 and F_2 are functional groups and A and B profile parts, F_1 and F_2 may be assumed to cooperate as a single group which defines the predominating component in the orientation pattern, as long as they are fairly close. In this component the profile is mainly determined by the largest profile group B. As A grows in size, cooperation may continue but since the largest profile part B decreases in size, the odor character may be expected to deteriorate or to weaken. Beyond a certain distance cooperation ceases and the functional group with the lowest solvation tendency becomes part of the profile (AF_2B) in



XXXV



XXXVI



XXXVII

the predominating orientation. Since the bulk of the molecule is now again available for the profile, the odor intensity may be expected to increase. This theoretical picture seems to be corroborated by the odor intensities of a series of macrocyclic oxa lactones (XXXI) described by Theimer and Davies (90). The parent compound, hexadecanolide (XXXII) is a powerful musk.

The oxa lactones with n = 2 to 4, representing the cooperative stage, have odors of intermediate intensity. At higher values of n, cooperation ceases and the oxa atom becomes part of the profile. Lactones with n = 5 and 6 have strong to very strong musk odors.

When F_1 and F_2 are identical, *e.g.*, in the macrocyclic diones (XXXIV) described by Ohloff *et al.* (77), the situation in the cooperative stage may be expected not to change. However, when both groups are located in opposite parts of the structure, each may govern its own oriented profile (AF_1B and AF_2B) or, if the configuration is favorable, F_1 and F_2 being sterically adjacent (XXXIII), we may expect deterioration of the odor. In the series XXXIV the dione with n = 3 is a musk whereas those with n = 5 and 6 are odorless.

Although stereoisomers of odorants are structurally closely related, their oriented profiles and, consequently, their odors may be vastly different. An interesting example has been described recently by Demole (38), who studied the stereochemistry of the hydrogenation products of the isomers obtained by condensation of camphene and phenol. Of all isomers and stereomers, only one, XXXV, was found to have a strong sandalwood odor. All others, particularly the stereomer XXXVI, have weak odors or are odorless.

The major interest of this example for the present subject is that the molecular shapes of XXXV and XXXVI do not differ much but the oriented profiles, viewed from the direction of the polar group, are vastly different. Another point of interest is that the constituents of sandalwood oil, α- and β-santalol, have partly flexible molecules, which may assume in the interaction stage relevant conformations, *e.g.*, XXXVII for β-santalol, resembling that of XXXV.

This brief discussion on stereomers leads us to a problem which has never received sufficient attention although it is probably one of the most important problems of research on olfaction, *i.e.*, the odor difference between optical antipodes.

The chirality (handedness) of substrate molecules is known to have a strong influence upon the effect of their interaction with enzymes and receptors. At first sight we would expect a similar influence upon the characteristics of olfactory response. Also in this case a stereospecific interaction between a substrate molecule and a component of the organism is involved, and although we know very little about nature and structure of the olfactory receptor sites, we may safely assume that they are proteinaceous molecules or part or combinations of such molecules (17) having a well defined chirality. Also, even in the absence of conclusive evidence, we have reasons to assume that the efficiency of olfactory interaction depends on the closeness of fit between the two partners in the interaction process, and if the steric requirements for participation in the latter were as critically important as they are in enzymatic processes, olfactory interaction could be expected to be highly specific with respect to the chirality of the odorant molecule.

However, an olfactory receptor site is probably able to accommodate a rather wide range of odorant structures with varying efficiency and the closeness of fit required for olfactory interaction seems to be a much cruder criterion than it is in the case of enzymatic processes. Also, we have seen that the nature of the functional group plays a less critical role in olfaction than it does in enzymatic processes.

Since enantiomers have the same scalar properties, physical as well as chemical, they offer a unique opportunity to isolate the aspect of oriented molecular shape from all other aspects. Unfortunately, research on olfaction has not made sufficient use of this opening, mainly because the principles involved have been imperfectly understood and because the requirements for experimental work are extremely critical.

In the first place asymmetry must be restricted to a single center, or, if more asymmetric centers are present, the chirality of all but one should be the same for both compounds. Diastereomers have entirely different configurations and consequently have different chemical, physical and physiological properties, including odors.

Secondly, any trace of impurity with low olfactory threshold, originating from different sources or different isolation techniques, may cause misleading odor differences. Consequently reliable results can only be obtained with enantiomers which have been prepared by resolution from the same racemic material under mild conditions and which have been purified with extreme care. Finally, trigeminal and taste effects must be avoided since they may cause perceptual differences which have no basis in olfaction. The safest way to exclude such effects is to study electrophysiological responses to stimulation of the olfactory membrane with enantiomers.

In most of the work published up to now these requirements are not fulfilled (22) and here we shall only mention the most reliable examples.

Doll and Bournot (41) measured threshold values for *d*- and *l*-menthol, prepared by resolution of the racemate and found 1.3 ± 0.5 μg per 1.5 *l* of air for both. Up to a concentration of 25.2 μg in 1.5 *l* of air no difference in the quality or intensity of the odor was observed, but at higher concentrations the cooling effect appears. The intensity of the latter increases more rapidly for *l*-menthol than for *d*-menthol whereas the quality of the total sensation remains the same.

Guillot *et al.* (55–57) resolved octanol-2 *via* the brucine salt of its monophthalate and measured the odor intensity of both enantiomers by panel evaluations of smelling strips dipped in solutions of increasing concentrations in ethanol, counting the positive, negative and uncertain answers. The ratio between the concentrations giving 50% positive answers for *l*- and *d*-menthol was found to be 3.2 to 3.3. No difference in odor quality was found. Stuiver (87) repeated these experiments, measuring threshold concentrations olfactometrically, and found a ratio of 2.9, which agrees well with Guillot's results.

Guillot also resolved isoborneol and obtained pure *l*-isoborneol and the *d*-enantiomer containing about 12% of *l*-antipode. Both have mainly a camphoraceous character but the secondary notes are different, *i.e.*, a celluloid note in *d*-isoborneol and a sweet and musty note for *l*-isoborneol. The intensity ratios for both notes were found to be different.

Rienäcker and Ohloff (84) prepared the two antipodes of β-citronellol by means of a two-step synthesis from (+)- and (−)-pinane. In this case the odor qualities were found to be different. No intensities or thresholds are mentioned in this paper.

It is interesting that there are several indications in the literature that the olfactory behavior of a racemic odorant differs from that of the antipodes. Naves (76) found no quality difference between the enantiomeric α-ionones but the threshold concentration of both seems to be 12 times higher than that of the racemic ionone. Veldstra (93) observed that the two liquid and practically odorless enantiomers of α-allylphenylacetic acid are practically odorless whereas the crystalline racemate has a honey odor. Provided these results can be confirmed, they demonstrate clearly that the interaction patterns of two enantiomers with the olfactory membrane are different since a mixture of equal parts of two identical compounds can only give the same information as the component parts, either directly or after any number of processing steps.

Electrophysiological work with enantiomers is still scarce. The electrophysiological threshold concentrations for the enantiomers and the racemate of the lure substance of the gypsy moth were found by Schneider (85) to be the same (10^{-2} μ) but the behavioral threshold for the (+) enantiomer was reported to be lower than for the racemate although Jacobson *et al.* (58) reported the same value for both.

III. SPECIFIC ANOSMIA

Specific anosmia is the inability to detect the odor of one or more odorants in persons with normal sensitivity for all others. Although the phenomenon may have a psychological basis it is more attractive and better in accordance with the observed facts to assume that specific anosmia is caused by the absence or inactivity in the olfactory membrane of one or more types of receptor site that are present and functioning properly in normal membranes.

If this explanation is correct, it enables us to predict roughly the results of a panel test for a group of odorants with closely similar odors. The odor relation between such odorants may be a case of pseudosimilarity, *i.e.*, it may not be based upon a similarity in the informational composition of the olfactory code message. In this case the anosmic patterns in the panel will not be the same for different odorants, *i.e.*, some panel members will be anosmic to one group of odorants and others to a different group.

If all odorants belong to the same "primary" class, *i.e.*, if the primary information pattern as well as the olfactory code message for such odorants consist mainly (but not exclusively) of one type of information, the anosmic pattern found for the panel will be the same or largely the same for all odorants of the group. In this case the evaluations obtained from the panel members can be classified in three types.

(a) "Normal" panel members will evaluate the odor sensation for all odorants in roughly the same way. For most, if not all, primary classes the odor will be found to be intensive and described in terms of identical or closely related key-words. Trained representatives of this type may also be able to distinguish the minor components in the olfactory code message from the major component.

(b) and (c) Anosmic panel members do not observe the major component. The minor components may or may not be observed, dependent on whether their frequency in the information pattern does or does not exceed the threshold required for the formation of a sensation. Consequently anosmic panel members can be expected to react in two ways to an odorant in the primary group.

(b) One type will describe the odorant as being odorless. In this case the minor components are not observed.

(c) The second type will describe the odor as weak or, in exceptional cases as moderately intensive, and the sensation will be expressed in terms of key-words totally unrelated to the key-words recorded by the normal group and not necessarily related to each other since the minor components are observed differently, depending on the specific acuities of individual observers.

Also, anosmic panel members will not be found to belong consistently to one of both anosmic types for the different representatives of a primary group of odorants since within such a group only the major component is the same but the minor components differ in nature as well as in frequency of occurrence.

Guillot (52, 53) described cases of specific anosmia for macrocyclic musks, steroid musks, hydrocyanic acid, ambergris, benzyl salicylate, methyl ionone, farnesol, and sclareol. The latter case is of particular interest since here, specific anosmia is a rule rather than an exception.

Guillot was also the first to observe cases of pseudosimilarity in olfaction. Although macrocyclic musks, steroid musks, and nitro musks have closely related characteristic odors, their structures are vastly different and seem to be related only by the rather rough criteria mentioned in the previous paragraph.

Guillot observed that the anosmic patterns between the musk subclasses seem to be independent. One of his subjects was anosmic to macrocyclic musks, a second one to steroid musks and a third one to both. However, the latter had a normal sensitivity for nitro musks. A similar independence was observed between the anosmias for hydrocyanic acid and benzaldehyde, both of which have similar bitter-almond odors.

Apart from specific anosmia, Guillot (54) described a few cases of parosmia, in which the odor sensation obtained widely differs from the normal one. In such cases the odors of the steroid musks are characterized as weak, fruity, floral, musky, or ionone-like notes. Another subject does not perceive the violet odor of the ionones and describes the odor as being woody. These may be interpreted on purely theoretical grounds as cases of the second type of anosmic observers (c) outlined above.

On the basis of these observations, Guillot concluded that a list of odorants for which specific anosmia has been observed may consist of representatives of the "odeurs fondamentales" and also that the identity of odor sensations caused by different odorants is not a criterion for identity of the sensory mechanisms involved.

Amoore *et al.* (10, 12, 16) also made use of specific anosmia as a tool to detect primary or fundamental odors. The work was based on a chance finding that one staff member showed specific anosmia for the sweaty odor of isobutyric acid. Two groups were carefully selected of which one (97 subjects) consisted of normal persons and a second one (10 subjects) consisted of people with specific anosmia to isobutyric acid. Both groups were presented with a series of buffered solutions of extremely pure isobutyric acid, the concentration of each solution being half that of the step before. For each concentration the ability to distinguish from an odorless liquid was carefully evaluated. The completely reproducible and highly significant result was that the difference between the mean thresholds for isobutyric acid of the normal and the anosmic groups was found to be 5.6 ± 0.4 binary steps.

With the same groups, the measurements were repeated with a large series of pure odorants that are in some way related to isobutyric acid, such as fatty acids with straight or branched chains, isobutyraldehyde, isobutyl alcohol, and isobutyl isobutyrate.

Isovaleric acid, which also has by far the lowest threshold value of the series, showed the highest threshold difference; and this result was interpreted by Amoore to mean that isovaleric acid is the compound closest to the typical primary odor.

Amoore (10) concluded that the receptor site involved in the sweaty primary odor is specialized to interact with the carboxylic group and that it has a wide tolerance since it is able to accommodate with fair efficiency the fatty acids with 4–7 carbon atoms and, less efficiently, acids of even smaller and larger chain length. Amoore found a strong positive correlation between the degree of anosmia and the amount of steric resemblance between each of the acids and isovaleric acid. This criterion was computed (9) from the length of 36 radii drawn in specific directions through the center of gravity of each of 3 perpendicular silhouettes of the molecular model. In a later paper (14) this tedious method was replaced by a computer technique according to which each of the three silhouettes is scanned successively with the same collection of over 4000 random lines, the intersections are counted and the molecular similarity is computed in logarithmic form.

In Amoore's work on fatty acids it is interesting to note that the threshold goes through a minimum at C_4 and C_5 and rises for acids with longer chains. This is usually explained by decreasing volatility with increasing molecular weight. However, beyond C_8, the threshold decreases again and the existence of two minima can hardly be explained on a physicochemical basis. Amoore (12) suggested that one type of receptor site is largely responsible for the detection of the C_4 and C_5 acids, while a second type may play the major role in the interaction pattern with C_9 and C_{10} acids.

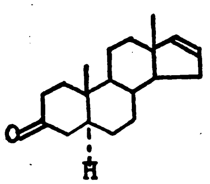
In his latest paper Amoore (13) listed 62 examples of specific anosmia collected as a result of a public request for such information published in two journals. This list, which may contain many duplicates, *i.e.*, examples representing the same "primary odor," is hoped to present the raw material for a more or less complete listing of primary or fundamental odors. By critical comparison of this collection of examples with earlier odor classification systems proposed in the literature, Amoore arrived at an estimate of 20 to 30 primary odors as a basis for continued experimental work.

There is little doubt that Amoore's systematic approach to specific anosmia holds every promise of becoming a rich source of information on structure-odor relations and on the various types of receptor site involved in olfactory interaction.

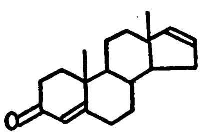
Recently, Beets and Theimer (24) described an interesting case of fundamental odor similarity between odorants belonging to two structurally unrelated types. The first type is represented by the steroid ketones XXXVIII and XXXIX, of which the odor had been described previously by Prelog *et al.* (82), the second one by the monocyclic ketone XL, discovered by Beets and van Essen (23).

In a preliminary test with a large panel, 51% of the observers could be classified as "normal" for all three ketones. This group described the odors by means of the same or closely related key-words of which urine, perspiration, sex, and animal were mentioned most frequently. To 6% the three ketones were odorless. A much larger group, 29%, could not observe the intensive urine note and described the odors by means of a wide range of key-words, totally unrelated to the descriptors used for the major component.

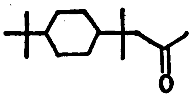
Although the remaining panel members gave inconsistent answers, the evalua-



XXXVIII



XXXIX



XL

tion patterns obtained for the ketones coincide to a very large extent and the conclusion seems to be justified that the intensive, urine-perspiration note for all three is derived from the same mono-osmatic component predominating in the primary information patterns and that, consequently, the three ketones belong to the same primary class. The oriented profiles of the molecular models show a strong similarity. The length is approximately equal but the steroid ketones are more bulky.

Although much work on specific anosmia remains to be done, the facts observed thus far seem to agree with the theoretical concepts outlined in the introduction to this paper.

IV. CROSS-ADAPTATION

As early as 1895, Zwaardemaker (99) suggested that olfactory cross-adaptation could be used as a tool to detect fundamental odor similarities. In recent years this suggestion has been followed by several authors.

Olfactory adaptation is the temporary loss of sensitivity as a result of continuous or repeated stimulation. When the conditioning and the tested stimuli are not identical, the word cross-adaptation is used.

Recent work of Adrian (2), Ottoson (78), and Stuiver (87) indicates that two superimposed components are involved in adaptation. The major one, characterized by fast recovery, is located in the olfactory bulb. Adrian attributed this effect, which may be compared with our inability to perceive, after some time, the ticking of a clock, to the loss of capacity of the olfactory signals to disorganize the resting activity of the bulb. A second, minor component characterized by slow recovery, seems to be located in the membrane. Since both components are directly or indirectly related to the structure of the primary information pattern, we may safely assume the existence of a correlation between the degree of cross-adaptation and the structural similarity of the information patterns involved.

Le Magnen (62) determined olfactory thresholds of the tested odorant before and after 10 min of exposure to the conditioning odorant. The difference between both threshold concentrations as a numerical expression of the degree of cross-adaptation was assumed to be directly related to the degree of similarity between the primary information patterns. Unfortunately, the materials used in this earlier work, comprising terpineol, vanillin, safrole, and tetralin, are structurally and olfactorily almost totally unrelated but the results indicate that even two information patterns representing widely different odorant structures usually have certain structural details in common. This is demonstrated more clearly in later work (63) on cross-adaptation with odorants belonging to the bitter-almond, the camphor, and the musk types.

After conditioning with benzonitrile, the odor of benzaldehyde is described as safrole-like while conditioning with a mixture of benzonitrile and safrole leaves an impression reminiscent of indole. The odor of benzaldehyde after conditioning with a mixture of safrole and indole is observed as benzene-like. Similar relations were found in a series of camphoraceous odorants.

Guillot's (52) conclusion, described in the previous section, that the relation-

TABLE 1

Conditioning Odorant	Tested Odorant			
	Isopropanol	Dioxan	Cyclopentanone	Cyclopentanol
Isopropanol	0.68	0.51	0.24	0.72
Dioxan	0.59	0.74	0.48	0.50
Cyclopentanone	0.18	0.54	0.67	0.04
Cyclopentanol	0.64	0.59	0.17	0.68

The numbers express the slope of the linear relation between the logarithms of the threshold concentration of the tested odorant and of the concentration of the conditioning odorant.

ship between certain subclasses of musk odorants is a case of pseudosimilarity, was confirmed by Le Magnen (63). Musk ketone and pentadecanolide, two musks with vastly different structures, showed no cross-adaptation.

The quantitative aspects of cross-adaptation were studied by Cheesman *et al* (31–33) for a group of 4 odorants. The results suggest a linear relationship between the logarithm of the threshold concentration of the tested odorant and the logarithm of the concentration of the conditioning odorant. Cheesman used the slope of this linear function as a measure of the degree of similarity between the olfactory information patterns of both odorants. The results are summarized in table 1.

The highest values are obtained when the tested and the conditioning stimuli are identical. These values are approximately independent (0.7) of the nature of the odorant within a single experiment.

Rather unexpectedly, high figures are obtained for 2 structurally dissimilar odorants (isopropanol and cyclopentanol) and low figures for odorants with closely related structures (cyclopentanol and cyclopentanone). However, comparison of the oriented profiles of molecular models, viewed in the direction of the dipole axis, shows that the profile similarity (percentage superimposability) of cyclopentanol and isopropanol is higher than that of cyclopentanol and cyclopentanone.

Engen (47) studied cross-adaptation effects in the series of straight-chain alcohols and was unable to detect a relationship between the magnitude of the effect and the chain length.

Köster (60) determined the effect of adaptation to a conditioning stimulus upon the percentage of positive answers obtained from a panel by exposure to the tested stimulus. His results were compared with the effects expected on the basis of Amoore's stereochemical theory. According to the latter, the interaction pattern of geraniol, which belongs to the floral primary class, should overlap with that of benzaldehyde but not with that of dioxan, which belongs to the ethereal class. Consequently, cross-adaptation can be expected between geraniol and benzaldehyde but not between geraniol and dioxan or between benzaldehyde and dioxan. In Köster's experiment the cross-adaptation effect between benzaldehyde and dioxan was extremely high while also geraniol affected the sensitivity for dioxan.

The available data on cross-adaptation are still extremely limited and mostly based on rather carelessly selected odorants. The observations described by Le Magnen (62, 63), Cheesman (31–33), and Köster (60) indicate that cross-adaptation can be used to detect the monoosmatic components of olfactory information patterns and that, in this way, it can supplement other techniques such as the analysis of specific anosmia discussed in the previous section.

V. ELECTROPHYSIOLOGICAL DATA

In previous sections we have discussed various methods to use characteristics of the verbal expression of odor sensations for the detection of fundamental similarities and for their correlation with structural similarities. This type of information has the advantage of being recorded from organisms functioning under normal conditions. Since it is recorded from the brain, beyond the terminus of the olfactory system, its disadvantage is clearly that it may be distorted by interaction with informational (psychological, subjective) elements of a different nature.

Olfactory information tapped from various parts of the olfactory system, the epithelium, the olfactory nerve, and the olfactory bulb, by means of electrophysiological techniques, can be used for the same purpose. Distortion by psychological factors is excluded in this case but on the other hand its reliability may be influenced by the fact that electrophysiological information is necessarily recorded from a system which is working under abnormal conditions.

Electrophysiology has contributed much to our knowledge of the functions and structure of the olfactory system. For these aspects, which are beyond the scope of this paper, and also for background information on electrophysiological techniques, the reader is referred to several extensive reviews (44, 73, 80).

However, the information concerning structure-response relations which can be derived from most of this work is limited. Much excellent research leading to important conclusions in other areas has no or little value for our insight into structure-response relations since it has been done with odd assemblies of odorants of unstated purity. For these reasons and also because this paper aims at the illustration of principles rather than at completeness, only a few of the most significant results are discussed in this section. More complete information will be found in a forthcoming review (22).

The activity in the receptor membrane can be recorded in three forms: the slow potential or electro-olfactogram (EOG), the activity in a small group of receptor units from an electrode in an arbitrary position in the membrane, and finally, the activity in a single receptor unit. Up to now, the latter has been possible only for the antennal receptors of insects and in a few other cases.

Ottoson (79) studied the amplitude of the electro-olfactogram, recorded from an electrode in contact with the mucosa of the frog, for a large series of odorants in aqueous solution. This technique has the disadvantage that the concentration of the odorant in the air-phase, defining the nonintrinsic component of response intensity is not simply related to the concentration in solution. Ottoson tried to reduce this effect as much as possible by using low concentrations on the linear part of the S-shaped curve expressing the relation between strength of stimulus and amplitude of response. Nevertheless the amplitudes in some cases are too closely related to volatility and concentration to be entirely convincing. This is particularly the case for the introduction of a hydroxyl group in propanol (propanediol-1,2) and in propionic acid (lactic acid), which reduces the amplitude to nearly zero, and for the high ratio (6) between the amplitudes obtained for salicylic aldehyde and 4-hydroxybenzaldehyde, which agrees well with the difference between their partial vapor pressure over an aqueous solution.

The influence of the concentration effect can be expected to be much less important for members of the same chemical series, and here the much more interesting intrinsic term of the response-intensity can be studied. Increasing unsaturation in the series propyl, allyl, and propargyl alcohol progressively increases the amplitude of the electro-olfactogram. Amplitudes obtained with the 4 isomeric butyl alcohols decrease in the sequence secondary, normal, iso-, and tertiary butanol. This order is different from those obtained by ranging the four alcohols according to decreasing vapor pressure, decreasing partial vapor pressure over an aqueous solution, and increasing steric accessibility of the functional group. In these cases the sequence is tertiary, secondary, iso-, and normal.

Ottoson (79) studied the amplitudes for the water soluble members of various homologous series, such as aldehydes, ketones and primary alkanols. In all cases amplitudes increased with chain length. For the primary alcohols this effect could be observed for aqueous solutions of members up to octanol, which showed a sharp amplitude increase over heptanol. However, for solutions in mineral oil the amplitude curve showed a maximum at C_7, after which the amplitude decreased sharply. This phenomenon may be related to Dethier's (39) discovery that the taste threshold for oil solutions is higher for octanol than for heptanol but that the reverse is true for aqueous solutions. Also Chadwick and Dethier (30), studying tarsal reception in blow flies, found a break in the curve at 7 carbons for both alkanols and alkanals. An explanation may be found in Dethier's hypothesis that water-soluble odorants stimulate *via* an aqueous phase and oil-soluble odorants *via* a lipid phase.

Ottoson (79) determined the concentrations of aqueous solutions of alkanols giving equal amplitudes of the electro-olfactograms. He found that for these concentrations thermodynamic activities (represented by the ratio between relative and saturated vapor pressures) of all members except the lowest 3 are roughly the same, and he suggested that for alcohols with 1 to 3 carbons, olfactory stimulation does not involve an equilibrium.

The electro-olfactogram is a summation effect of the response of the whole membrane. More specific information concerning responses of receptors can be expected from micro-electrode recordings from small groups of cells or, even more so, from single cells in insect antennae.

However, before discussing examples of such recordings, we should realize that the receptor site interacting more or less efficiently with an odorant molecule, must have molecular dimensions, and that consequently even the recording obtained from a micro-electrode in a single cell is a gross summation effect of the responses of different types of site, represented in various frequencies on the membrane of the cell. This does not exclude the possibility of obtaining information on selective sensitivities of receptor sites, since different combinations of such sites can be expected to respond differently. The information obtained will become increasingly selective with decreasing complexity of the recording area.

Indications of receptor selectivity were obtained by Gesteland *et al.* (51) by recording slow potentials and action spikes from low-impedance extracellular electrodes in the epithelium of the frog. On the basis of their results the authors attempted a rough classification in 8 overlapping groups of electrode locations in terms of odorants for which responses were obtained. This classification does not offer any clue to response-structure relations although it does resemble slightly Amoore's classification in primary types.

The olfactory receptors on insect antennae offer unique opportunities for electrophysiological recordings from single receptor units. In the hands of Schneider and his co-workers (25–28, 61, 85, 86) this led to an interesting demonstration of the chemical selectivity of olfactory receptors. All units tested by this group have a reproducible spectrum of odor stimuli to which they respond. On the basis of these spectra, cells can be classified as "odor generalists," such as the basiconical cells of the moth and the placoid sensilla of the honey bee, which show unique although overlapping spectra, and "odor specialists," such as the sex-attractant sensitive receptors of the male moth, which give stereotyped responses to a limited number of odorants (28).

In recordings from single units (61), the response frequency often shows a phasic-tonic course, *i.e.*, it rises almost instantaneously to a maximum and drops rapidly to an approximately constant level. Both the height of the maximum and that of the stationary level can be used for characterization of the response. The unit can respond to stimulation either by an increase of impulse frequency (excitation) or by a decrease (inhibition). A third possibility is that the unit does not respond. The question whether inhibition is an intrinsic component of the olfactory information pattern is still undecided (25).

Lacher (61) recorded the impulses from a series of placoid sensilla on the antenna of the honey bee during stimulation with a number of odorants. Different response spectra were recorded from 5 or 6 receptor units for the members of homologous series of aldehydes and acids. Lacher extended this work to the recording of response spectra from 47 units for 33 odorants. Although the number of units from which responses were recorded (17 out of 3,000 pro antenna for the worker bee and 30 out of 15,000 for the drone) can hardly be representative for the total spectrum of the antenna, an attempt can be made to detect correlations even between such fragments of the spectrum and odorant structures. This may be done by comparing the trends of profile similarities between pairs of odorants with those of response spectrum similarities for the same pairs. For the spectra published by Lacher no correlation between these two trends could be found (22) when common inhibitory, excitatory and zero responses were counted, but when only excitatory responses were considered, the trends of profile similarity and spectrum similarity had the same direction in the majority of cases. The very limited material does not justify any conclusion but the method may become a useful tool as soon as recordings from larger numbers of units become available, expecially in combination with Amoore's (14) technique for the quantitative measurement of profile similarities. Also, in this type of work a more expert selection of odorants would be desirable.

Schneider *et al.* (86) and Boeckh *et al.* (28) published similar spectra for the antenna of the Asiatic silk moth. The first mentioned authors also attempted to characterize the response pattern for each odorant by means of the ratio between the numbers of positively and negatively responding units. No relation could be found between this ratio and odorant structure.

Boeckh (26, 27), recording from the "grass-sensitive" receptors on the antenna of the migratory locust, studied responses to stimulation with series of odorants with straight, saturated carbon chains and different functional groups. In the C_6 and C_7 series only the acids were more or less active, alcohols and aldehydes being inactive. Introduction of a double bond changes this picture; 2-hexenol, 2-hexenal, and 2-hexenoic acid were all active. In the series of saturated fatty acids, formic and acetic acid are inactive, after which the activity (number of impulses in 500 msec.) increases gradually. Caproic acid has the highest activity of the series. This agrees well with the fact that caproic acid has the lowest electrophysiological threshold (10^9 molecules/cm^3 of air) of the acid series. A still lower threshold (10^8 molecules/cm^3) was found for 2-*trans* hexenal. The electrophysiological threshold is defined by Boeckh as the lowest concentration which still gives a nerve impulse reaction, different from the control. The activity decreases and the length of the oriented profile viewed in the direction of the dipole axis increases in the order *trans*-hexenal, caproic acid, *cis*-hexenol, hexanol.

Schneider's (85) work on electrophysiological and behavioral thresholds of the 4 stereomers of bombycol, the sex attractant of *Bombyx mori*, demonstrates the high stereospecificity of the specialist receptor units (table 2). The low electrophysiological threshold shows the very significant preference for the bent shape of the bombycol molecule. In the behavioral thresholds this preference seems to be faintly reflected in the difference between that of the *cis-trans* isomer, which also has a bent profile, and those of the *cis-cis* and *trans-trans* isomers, which both have fairly straight profiles.

TABLE 2

Hexadecadiene-10-12-ol-1		Behavioral Threshold	Electrophysiological Threshold
10	12		
		μg/ml	*μg*
trans	*cis* (bombycol)	10^{-12}	10^{-10}
cis	*trans*	10^{-3}	10^{-5}
cis	*cis*	1	10^{-6}
trans	*trans*	1	10^{-5}

Up to now recordings from olfactory nerve twigs have yielded relatively little information on structure-odor relations.

Mozell (74), recording from two widely separated twigs of the frog, found different response magnitude ratios and different time lapses between the two nerve responses for different odorants. Later, Mozell (75) extended this work for a series of 4 odorants in 6 different concentrations and determined for both criteria the number of animals (out of 10) in which the value found falls into a given rank (1 to 4) at each concentration. None of the chemicals showed ratios confined to a single rank in all concentrations but the order was independent of concentration.

Structurally related pairs of odorants such as geraniol and citral or limonene and octane were always found in neighboring ranks. For latency differences the internal consistency of the data, which was weak at low concentrations, increased with concentration. These results suggest that the topology of the epithelium and perhaps also temporal elements are represented in the olfactory information pattern.

The topological aspect of olfactory information is also indicated by the work of Gesteland *et al.* (50), who recorded the activity in small bundles of axons immediately underneath the basal membrane. An odorant may increase, decrease or not affect the chaotic resting activity. The authors observed that two odorants that cause similar sensations in the human nose, such as menthol and menthone or benzonitrile and nitrobenzene, produce similar responses in one electrode location, but that it is always possible to find a location in which the units respond differently. This is as expected since even two odorants that are closely related structurally cannot produce identical olfactory information patterns. On the basis of this work, the authors offered some interesting speculations on olfactory discrimination, which will not be discussed in this paper, in the course of which the possibility of a holographic structure of the primary information pattern is mentioned.

A holographic organization of the olfactory information is particularly attractive since we have noticed in some cases rough but significant correlations in response spectra obtained from small numbers of units which cannot be representative of the whole population of receptor units. Also we know that abnormal secretion of mucus on the epithelium may weaken or destroy the sense of smell but never distorts it. As long as we can smell at all, the odor impression produced by an odorant never changes into a different type. These phenomena suggest that the olfactory system and the brain are able to construct from the primary information pattern received from any part of the stimulated epithelium, even a small part, an odor impression having all the characteristics of the one based on the information pattern of the whole receptor area although it may be weaker and show fewer details. This could mean either that the primary information pattern consists of a very large number of areas with identically-structured information or that it has holographic characteristics.

The first indications that, also on the level of the olfactory bulb, topological and temporal elements are intrinsic parts of the olfactory information package were obtained by Adrian (1, 3). In hedgehogs, rabbits and cats, he observed that stimulation of the receptors with esters and ketones gives responses predominantly in the oral part of the bulb whereas stimulation with hydrocarbons is recorded mainly in the aboral part. Also the temporal pattern of responses was related to odorant structure. The posterior part of the bulb of the cat responds particularly to stimulation with decayed meat, fish and trimethyl amine.

The activity in mitral cells consists frequently of one series of large uniform spikes and a number of smaller ones of varying size. Adrian (4) explained the large spikes as discharges of one mitral cell and the smaller ones as belonging to neighbouring units leading to other groups of receptors. He could nearly always find an odorant that in low concentration gave only the large spike discharge. On the basis of this technique Adrian (5, 6) later attempted a classification of bulb units into 5 main groups which respond especially but not exclusively to stimulation of the epithelium with each of 5 classes of odorant: aromatic hydrocarbons, esters, alkanes, terpenes and sulfur compounds. Adrian assumed that the receptors in the epithelium are arranged in small zones, each with a special affinity to a group of odorant structures.

Moulton (67–70) and Moulton and Tucker (73) studied multispike discharges in the secondary neurones of the bulb of the rabbit in which six electrodes are permanently implanted. In this way bar diagrams are obtained which are more or less characteristic for each odorant. However, a number of 6 sites out of perhaps 10^3 to 10^4 cannot be representative of the whole population of sites and may reveal similarities that disappear when the number of recording sites is extended. This is indicated by the fact that structurally unrelated odorant pairs such as benzothiazole and anisole have similar patterns, while structurally related pairs such as cyclopentadecanone and pentadecanolide have different response patterns. Odorants with similar response patterns can often be distinguished by the different time course of the responses.

Statistical analysis of electrophysiological data, developed by Döving (42), promises to become a useful tool in research on response-structure relations since its results can be related with those of psychological methods and with profile parameters of odorant molecules.

Recordings were made from 85 bulbar units of the frog for a series of 5 stimulants consisting of *l*-menthol, coumarin, salicylic aldehyde, an impure mixture of nerol and geraniol, and a mixture of the two stereomers of citral. The response patterns obtained, consisting of excitatory, inhibitory, and zero responses, were statistically processed by means of a chi-square test for pairs of odorants, starting from the assumption of odor independence within each pair. The chi-square values can be used as a criterion for the degree of similarity between the information patterns of the two odorants.

The assumption of pattern independence was rejected statistically for citral/geraniol and for citral/*l*-menthol. By far the highest value was found for the first of these pairs. This is comprehensible since the two *cis*-isomers as well as the two *trans*-isomers have closely related profiles whereas only one of the stereomers in citral can assume a conformation with a profile somewhat similar to that of menthol. The response patterns of coumarin, geraniol and salicylic aldehyde were

TABLE 3

Odor Pair	Mean Similarity Estimate	Mean Confusibility	Chi-square
	%	%	
Menthol/salicylic aldehyde	30	6	0.22
Menthol/citral	40	19	4.93
Menthol/geraniol	59	24	3.76
Salicylic aldehyde/acetophenone	64	55	10.42
Salicylic aldehyde/anisole	56	48	0.24
Salicylic aldehyde/citral	37	14	2.12
Salicylic aldehyde/geraniol	31	10	1.41/0.34*
Acetophenone/anisole	51	72	6.29
Acetophenone/geraniol	42	24	0.23
Anisole/geraniol	38	12	4.67
Citral/geraniol	84	82	9.19
Camphor/tert·butanol	52	36	5.70
Camphor/tert·pentanol	67	56	0.54
Camphor/3-methyl-butanone-2	62	28	10.04
tert Butanol/tert pentanol	80	70	15.86
tert Butanol/3-methyl-butanone-2	54	42	13.57
tert Pentanol/3-methyl-butanone-2	61	45	8.47

* Values obtained from two different experimental series.

independent although all three are representatives of Amoore's floral primary class.

Later, Döving (43, 45) extended this work to 33 odorants belonging to the 7 primary groups proposed in Amoore's earlier papers. In general the impression is obtained that the values found for pairs of odorants are significantly correlated with the degree of profile similarity and rather independent of their classification in the system of primary groups. Of the 5 classes studied by Döving only the musks show sufficient homogeneity in their bulbar response patterns to justify their grouping as a primary class.

Döving and Lange (46) studied the correlation between the chi-square values obtained with Döving's technique and the values resulting from two psychological methods (judgements of similarity and measures of confusion). The results are summarized in table 3.

The agreement between the two psychological methods was found to be better than between each of these methods and the chi-square method. Since the latter operates on the bulbar level in the frog and both psychological techniques on the brain level in man, this is not surprising. With some notable exceptions the trends between the pair-values in table 3 agree with the trends of profile similarity estimates. The high value found for the seemingly heterogeneous pair camphor/methyl butanone is of interest since both have in common the part of their molecular profile surrounding the functional group.

VI. INTENSITY OF RESPONSE

In the previous paragraphs we have hardly mentioned the quantitative aspect of olfactory response, its intensity. It consists of a nonintrinsic term, which is determined by the odorant concentration in the carrier phase, and an intrinsic term, which is a function of molecular structure.

Information on intrinsic intensity can be obtained by measuring the minimal concentration (threshold) at which a significant or, depending on definition, an identifiable olfactory response can be obtained.

Most values of threshold concentrations reported in the literature have been determined in the conventional way with people or, on the basis of behavior, with animals. Such data are of limited interest for the subject of this paper since there exists no simple, known relation between the odorant concentration in the air current entering the nose and the concentrations in the carrier phase, *i.e.*, the availability of odorant molecules for interaction with the receptor sites. One of the factors involved is the heavy loss of odorant molecules in the nasal cavity, owing to trapping by the mucous linings. Stuiver (87) calculated that approximately 2% of the molecules in the air current entering the nose reach the epithelium. Many threshold values measured by conventional methods may show regularities which are related only partly to olfaction and mainly to the relation between the solubility of the odorant molecules in water and the degree in which they are trapped before reaching the receptor sites. An example may be Stuiver's (87) observation that the intramolecularly chelated and consequently less water-soluble ortho nitrophenol has a much lower threshold (5.0×10^9) than the two other isomers (*ca.* 10^{13}).

Thresholds determined with conventional methods can be applied only for comparing closely related odorants such as homologs for which the effect of trapping can be assumed to be approximately equal. An example is the work of Moulton and Eayrs (72), who determined behavioral thresholds in male rats for the homologous alkanols of 1 to 12 carbon atoms and found a linear relationship between the logarithms of threshold concentration and chain length.

The unknown influence of trapping can be avoided by the use of techniques involving a direct contact between the air current carrying the odorant and the receptors. Much of the electrophysiological work as well as the study of behavioral thresholds with insects satisfies this requirement. An example of the latter is the work of Dethier and Yost (40) who determined the concentration of homologous alcohols in the vapor phase rejected by 50% of a population of blow flies. Also in this case a linear relationship was observed between the logarithms of the rejection threshold and the number of carbon atoms. When the threshold concentrations are expressed in thermodynamic activities, the intermediate terms roughly obey the rule of equal stimulating effectiveness at equal thermodynamic activity.

Theoretically we may expect the structure of the primary information pattern to change as the concentration of the odorant in the carrier phase decreases and approaches the threshold value. We can assume that this pattern is a complex assembly of contributions from a large number of types of interaction complexes between odorant molecules and receptor sites. In this assembly one type may quantitatively predominate, and to the extent to which this is the case, we approach the nonexistent ideal of the mono-osmatic odorant.

This type of response pattern consisting of a major component and various types of minor components is represented by odorants belonging to true primary classes. As the concentration of such an odorant decreases, the minor components can be expected to fade away before the major component, and the complexity which tends to blur the picture at higher concentrations is gradually eliminated. This means that for odorants of the same primary type, the threshold value could very well be a quantitative criterion for a true mono-osmatic phenomenon. On the basis of this philosophy the existence of simple relations between threshold values within a primary class is possible. Such relations might present a new tool for the detection and the definition of the scope of true primary classes. Also, the study of response spectra at close-to-threshold concentrations, *e.g.*, by electrophysiological means, may be particularly instructive since they are less complex and can be interpreted more easily than those obtained at higher concentrations.

VII. INDIRECT RELATIONS

Since the molecular structure of an odorant is the common origin of all its properties, these properties are necessarily interrelated. Although the nature of this interrelationship may be extremely intricate to the extent that it escapes detection, it does at least present the theoretical possibility of discovering meaningful links between olfactory information and one or more other characteristics of the odorant such as physical and chemical properties or geometrical parameters of molecular structure. In the earlier days of olfactory research, several attempts have been made to detect such relations. In this paragraph we will limit the discussion to two modern examples.

Wright (95) suggested studying spectral regions which are known to be associated with molecular configuration. He actually found some frequencies in the Raman spectra below 1000 cm^{-1} which a number of chemicals with bitter-almond odor seemed to have in common. Later he (97) studied the far infrared spectra between 80 and 340 cm^{-1} of 45 musks and 15 chemicals which are structurally related to the musks although they do not possess a musk odor. He concluded that, in addition to other criteria such as adequate volatility, one or more functional groups to give the molecule the correct orientation at the receptor membrane and a minimum molecular size, the musk odorant is characterized by three vibration frequencies near 100, 160, and 260 cm^{-1} in its spectrum.

The statistical significance of the correlations described by Wright has been criticized by Davies (35). His arguments could be interpreted to confirm the view mentioned in previous paragraphs that nitro musks and macrocyclic musks are two distinct subareas in the wide territory of the musk odor.

Wright (96) also studied the far-infrared spectra of chemicals with bitter-almond and cuminic odors. According to Wright and Robson (98) the bitter-almond odor is characterized by frequencies near 175, 225, and 345 cm^{-1} while the cuminic odor is associated with frequencies near 175, 265, and 310 cm^{-1}. A peak near 395 cm^{-1} is shared by both groups.

On the basis of a theory on the mechanism of odorant-receptor interaction and stimulus formation, proposed by Davies (34), Theimer and Davies (90) studied a large number of musks belonging to various structural classes. According to these authors molecular morphology in the musk-class is characterized by a cross-sectional area of the oriented molecule in the range of 40 to 57 $Å^2$ and by a length-over-breadth ratio of 2.8 to 3.3. The odor intensity of musks is governed by a third, purely empirical, requirement, namely, that the desorption rate from a water surface should lie in the range 0.4 to 1.7.

Similar criteria were defined by Davies for the nitro musks (37) and for amber odorants (36).

VIII. CONCLUSIONS

The essential stages of the process of olfaction are the interaction, the formation of the primary information pattern, its processing in the receptor cell and in the olfactory bulb, and the delivery of the final product, the olfactory code message, to the brain.

Although the information on structure-response relations available at the present stage and reviewed in this paper shows large gaps, it does not contradict and often supports the tentative concepts on olfactory interaction postulated in the introduction. We may now ask the question what we can say about the informational aspects of the process.

Two partners are involved in olfactory interaction, a population of stimulant molecules and a population of receptor sites. There is little doubt that the major criteria for the first of these partners are the statistical composition of the orientation pattern at the receptor surface and the morphology of the molecular profiles in the predominating orientations.

All we can safely assume about the second partner is that the receptor sites are located on proteinaceous molecules, that they have molecular dimensions, that the number of types is probably limited, and that their ability to accommodate sterically the oriented profiles of arriving odorant molecules is directly related to the efficiency of the interaction.

The transitory population of interaction complexes which is the immediate result of the interaction produces a heterogeneous population of elementary bits of information which we have called the primary information pattern.

We know little or nothing about the processing of elementary bits of information taking place in the olfactory cell but we are fairly certain that the information element emerging from each receptor cell retains its individuality up to the level of the olfactory bulb. It seems reasonable to assume that this direct projection of the membrane topology onto the bulb has a function, that the topological characteristics are retained in the processing steps taking place in the glomerules and the mitral cells, and that they are represented in the olfactory code message which is delivered to the brain. On the basis of this reasoning we may venture to formulate as a working hypothesis that the informational content of the element emerging from each receptor cell consists not only of an intensity factor in which amplitude as well as frequency may be represented, but also of a topological factor. The latter is probably associated with odor quality.

The structure of the information contained in the primary information pattern seems to be identical with that of any fragment of this pattern. Only the resolution of its details decreases with the size of the fragment. Possible explanations, *i.e.*, a holographic arrangement or a repetition of areas with identical information characteristics, have been mentioned in the text. Anyhow, we can be certain that the distribution of site types over the epithelium is not random. Probably differentiation between cells is due to the fact that the receptive membranes of the receptor cells differ in the nature and in the numerical ratio of their site types.

The speculative nature of these few thoughts on olfactory information is characteristic of the whole area of structure-response relations. There is hardly an aspect which can be discussed without walking the razor's edge between speculation and facts. The obvious reason for this is that the facts are sparse. Most of them have been obtained as a by-product of olfactory research in general. In many excellent papers, a few simple additional experiments, which would have presented the basis for a more positive conclusion, are lacking because the range of vision of the author was strictly limited to his own discipline.

The slowly increasing interdisciplinary cooperation of recent years holds every promise of accelerating progress. In order to realize this the practice of using unidentifiable mixtures or chemicals of doubtful purity or extremely low molecular weights should be discontinued. Only experiments for which odorant structures have been planned as carefully and as expertly as other aspects can be expected to yield a maximum of information. The realization of this requirement may present some organizational problems since most disciplines involved are far removed from chemistry, physics and pharmacology.

Other particularly important targets for future research include the development of methods for the numerical expression of oriented profile parameters, a more extensive study of response differences between pure enantiomers, measurement of electrophysiological response spectra for larger numbers of units and for well chosen stimulants, and, finally, further exploration of promising techniques such as threshold values, cross-adaptation, and specific anosmia.

There is little doubt that proper organization and cooperation in this area will remove most of the shadows in a single decade.

REFERENCES

1. Adrian, E. D.: Sensory discrimination. Brit. Med. Bull. 6: 330–333, 1949/1950.
2. Adrian, E. D.: The electrical activity of the mammalian olfactory bulb. Electroencephalogr. Clin. Neurophysiol. 2: 377–388, 1950.
3. Adrian, E. D.: Olfactory discrimination. L'Année Psychol. 50: 107–113, 1951.
4. Adrian, E. D.: Sensory messages and sensation. Acta Physiol. Scand. 29: 5–14, 1953.
5. Adrian, E. D.: The action of the mammalian olfactory organ. J. Laryng. Otol. 70: 1–14, 1956.
6. Adrian, E. D.: Des réactions électriques du système olfactif. Actual. Neurophysiol. 1: 11–18, 1959.
7. Amoore, J. E.: The stereochemical specificities of human olfactory receptors. Perf. Ess. Oil. Rec. 43: 321–330, 1952.
8. Amoore, J. E.: The stereochemical theory of olfaction. 1. Identification of the seven primary odors. Proc. Sci. Sect. Toilet Goods Ass. 37 (suppl.): 1–12, 1962.
9. Amoore, J. E.: Psychophysics of odor. Cold Spring Harbor Symp. Quant. Biol. 30: 623–637, 1965.
10. Amoore, J. E.: Specific anosmia: a clue to the olfactory code. Nature (London) 214: 1095–1098, 1967.
11. Amoore, J. E.: Stereochemical theory of olfaction. *In* Chemistry and Physiology of Flavors, ed. by H. W. Schultz, E. A. Day and L. M. Libbey, Avi Publishing Co., Westport, 1967.
12. Amoore, J. E.: Specific anosmias and primary odors. Proceedings of the Nato Summer School, Istanbul, 1968, pp. 71–85.
13. Amoore, J. E.: A plan to identify most of the primary odors. *In* Olfaction and Taste, vol. III, Ed. by C. Pfaffman, to be published.
14. Amoore, J. E., Palmieri, G. and Wanke, E.: Molecular shape and odour: Pattern analysis by PAPA. Nature (London) 216: 1084–1087, 1967.
15. Amoore, J. E. and Venström, D.: Correlations between stereochemical assessments and organoleptic analysis of odorous compounds. *In* Olfaction and Taste, vol. 2, ed. by T. Hayashi, Pergamon Press, Oxford, 1967.

16. Amoore, J. E., Venström, D. and Davis, A. R.: Measurement of specific anosmia. Percept. Mot. Skills 26: 143–164, 1968.

17. Ash, K. O. Chemical sensing: an approach to biological molecular mechanisms using difference spectroscopy. Science 162: 452–454, 1968.

18. Beets, M. G. J.: Structure and odour. *In* Molecular Structure and Organoleptic Quality, Society of Chem. Ind., London, 1957.

19. Beets, M. G. J.: A molecular approach to olfaction. *In* Molecular Pharmacology, vol. II, ed. by E. J. Ariëns, Academic Press, New York, 1964.

20. Beets, M. G. J.: Les muscs. Structure et odeur. La France et ses parfums, 1967: 113–122.

21. Beets, M. G. J.: Odor and molecular structure. Olfactologia 1: 77–92, 1968.

22. Beets, M. G. J.: Olfactory response and molecular structure. *In* Handbook of Sensory Physiology, vol. IV, ed. by L. M. Beidler, Springer Verlag, New York, to be published, 1970.

23. Beets, M. G. J. and Essen, H. van: U.S. patent application 849.192, 1969.

24. Beets, M. G. J. and Theimer E. T.: Odor similarity between structurally unrelated odorants. *In* Ciba Foundation Symposium on Mechanisms of Taste and Smell in Vertebrates, London, September 1969, to be published.

25. Boeckh, J.: Elektrophysiologische Untersuchungen an einzelnen Geruchsrezeptoren auf den Antennen des Totengräbers (Necrophorus, Coleoptera). Z. Vergl. Physiol. 46: 212–248, 1962.

26. Boeckh, J.: Reaktionsschwelle, Arbeitsbereich und Spezifizität eines Geruchsrezeptors auf den Heuschreckenantenne. Z. Vergl. Physiol. 55: 378–406, 1967.

27. Boeckh, J.: Odor specificity and reaction range of a single olfactory receptor cell. Proceedings of the Nato Summer School, Istanbul, 1968, pp. 213–224.

28. Boeckh, J., Kaissling, K. E. and Schneider, D.: Insect olfactory receptors. Cold Spring Harbor Symp. Quant. Biol. 30: 263–280, 1965.

29. Braun, J. von and Kröper, H.: Geruch und Konstitution (1e Mitteilung). Ber. Deut. Chem. Ges. 62: 2880–2885, 1929.

30. Chadwick, L. E. and Dethier, V. G.: Stimulation of tarsal receptors of the blowfly by aliphatic aldehydes and ketones. J. Gen. Physiol. 32: 445–452, 1949.

31. Cheesman, G. H.: Odour and chemical constitution— a new approach. Proc. Roy. Aust. Chem. Inst. 27: 70–73, 1960.

32. Cheesman, G. H. and Mayne, S.: The influence of adaptation on absolute threshold measurements of olfactory stimuli. Quart. J. Exp. Psychol. 5: 22–30, 1953.

33. Cheesman, G. H. and M. J. Townsend: Further experiments on the olfactory thresholds of pure chemical substances, using the "sniff-bottle method." Quart. J. Exp. Psychol. 8: 8–14, 1956.

34. Davies, J. T.: L'Odeur et la morphologie des molécules. Ind. Parf. 8: 74–79, 1953.

35. Davies, J. T.: Olfactory theories. *In* Handbook of Sensory Physiology, vol. IV, ed. by L. M. Beidler, Springer Verlag, New York, to be published, 1970.

36. Davies, J. T.: Personal communication, 1965.

37. Davies, J. T.: Personal communication, 1968.

38. Demole, E.: Synthèses et relations entre constitution chimique et odeur dans la série des terpényl-3-cyclohexanols. Helv. Chim. Acta 47: 1766–1774, 1964.

39. Dethier, V. G.: Taste sensitivity to homologous alcohols in oil. Fed. Proc. 11: 34, 1952.

40. Dethier, V. G. and Yost, M. T.: Olfactory stimulation of blowflies by homologous alcohols. J. Gen. Physiol. 35: 823–839, 1952.

41. Doll, W. and Bournot, K.: Über den Geruch optischer Antipoden. Schimmel Ber. 1948: 150–158.

42. Döving, K. B.: Studies on the responses of bulbar neurons of the frog to different odour stimuli. Rev. Laryngol. 1965 (suppl.): 845–854.

43. Döving, K. B.: Analysis of odour similarities from electrophysiological data. Acta Physiol. Scand. 68: 404–418, 1966.

44. Döving, K. B.: Problems in the physiology of olfaction. *In* The Chemistry and Physiology of Flavors, ed. by H. W. Schultz, E. A. Day and L. M. Libbey, Avi Publishing Co., Westport, 1967.

45. Döving, K. B.: Electrophysiological studies on sensory relatedness. Proceedings of the Nato Summer School, Istanbul, 1968, pp. 493–508.

46. Döving, K. B. and Lange, A. L.: Comparative studies of sensory relatedness of odours. Scand. J. Psychol. 8: 47–51, 1967.

47. Engen, T.: Cross-adaptation to the aliphatic alcohols. Amer. J. Psychol. 76: 96–102, 1963.

48. Farbman, A. I.: Structure of chemoreceptors. *In* Chemistry and Physiology of Flavors, ed. by H. W. Schultz, E. A. Day and L. M. Libbey, Avi Publishing Co., Westport, 1967.

49. Gasser, H. S.: Olfactory nerve fibers. J. Gen. Physiol. 39: 473–496, 1956.

50. Gesteland, R. C., Lettvin, J. Y. and Pitts, W. H.: Chemical transmission in the nose of the frog. J. Physiol. (London) 181: 525–559, 1965.

51. Gesteland, R. C., Lettvin, J. Y., Pitts, W. H. and Rojas, A.: Odor specificities of the frog's olfactory receptors. *In* Olfaction and Taste, vol. 1, ed. by Y. Zotterman, Pergamon Press, Oxford, 1963.

52. Guillot, M.: Anosmies partielles et odeurs fondamentales. C. R. Hebd. Séances Acad. Sci. (Paris) 226: 1307–1309, 1948.

53. Guillot, M.: Sur quelques caractères des phénomènes d'anosmie partielle. C. R. Séances Soc. Biol. 142: 161–162, 1948.

54. Guillot, M.: Anosmies et parosmies individuelles spécifiques. Recherches 1953 (October): 26–31.

55. Guillot, M.: Pouvoir rotatoire et olfaction. Recherches 1955 (May): 24–31.
56. Guillot, M. and Babin, R.: Pouvoir rotatoire et odeur. C. R. Hebd. Séances Acad. Sci. (Paris) 229(II): 1363–1365, 1969.
57. Guillot, M. and Thibaut, P.: Influence du pouvoir rotatoire sur la qualité de l'odeur. C. R. Hebd. Séances Acad. Sci. (Paris) 232(I): 1138–1140, 1951.
58. Jacobson, M., Beroza, M. and Jones, W. A.: Isolation, identification and synthesis of the sex attractant of the gypsy moth. Science 132: 1011–1012, 1960.
59. Kaissling, K. E. and Renner, M.: Antennale Rezeptoren für Queen substance und Sterzelduft bei der Honigbiene. Z. Vergl. Physiol. 59: 357–361, 1968.
60. Köster, E. P.: Adaptation, recovery and specificity of olfactory receptors. Rev. Laryngol. 1965 (suppl.): 881–894.
61. Lacher, V.: Elektrophysiologische Untersuchungen an einzelnen Rezeptoren für Geruch, Kohlendioxyd, Luftfeuchtigkeit und Temperatur auf den Antennen der Arbeitsbiene und der Drohne (Apis Mellifica L). Z. Vergl. Physiol. 48: 587–623, 1964.
62. Le Magnen, J.: Étude d'une méthode d'analyse qualitative de l'olfaction. L'Année Psychol. 43/44: 249–264, 1942/1943.
63. Le Magnen, J.: Analyse d'odeurs complexes et homologues par fatigue. C. R. Hebd. Séances Acad. Sci. (Paris) 226: 753–754, 1948.
64. Lorenzo, A. J. D. de: Studies on the ultra structure and histophysiology of cell membranes, nerve fibers and synaptic junctions in chemoreceptors. *In* Olfaction and Taste, vol. 1, ed. by Y. Zotterman, Pergamon Press, London, 1963.
65. MacLeod, P.: Histologie et physiologie du récepteur olfactif. La France et ses Parfums 6: 233–237, 1963.
66. Martin, A. J. P.: Ciba Foundation Symposium on Mechanisms of Taste and Smell in Vertebrates. London, September, 1969, to be published.
67. Moulton, D. G.: Electrical activity in the olfactory system of rabbits with indwelling electrodes. *In* Olfaction and Taste, vol. 1, ed. by Y. Zotterman, Pergamon Press, Oxford, 1963.
68. Moulton, D. G.: Differential sensitivity to odors. Cold Spring Harbor Symp. Quant. Biol. 30: 201–206, 1965.
69. Moulton, D. G.: Spatio-temporal patterning of response in the olfactory system. *In* Olfaction and Taste, vol. 2, ed. by T. Hayashi, Pergamon Press, Oxford, 1967.
70. Moulton, D. G.: Electrophysiological and behavioral responses to odor stimulation and their correlation. Olfactologia 1: 69–75, 1968.
71. Moulton, D. G. and Beidler, L. M.: Structure and function in the peripheral olfactory system. Physiol. Rev. 47: 1–52, 1967.
72. Moulton, D. G. and Eayrs, J. T.: Studies on olfactory acuity. II. Relative detectability of *n*-aliphatic alcohols by the rat. Quart. J. Exp. Psychol. 12: 99–109, 1960.
73. Moulton, D. G. and Tucker, D.: Electrophysiology of the olfactory system. Ann. N.Y. Acad. Sci. 116: 380–428, 1964.
74. Mozell, M. M.: Olfactory discrimination; electrophysiological and spatiotemporal basis. Science 143: 1336–1337, 1964.
75. Mozell, M. M.: The effect of concentration upon the spatiotemporal coding of odorants. *In* Olfaction and Taste, vol. 2, ed. by T. Hayashi, Pergamon Press, Oxford, 1967.
76. Naves, Y. R.: Sur le dédoublement de la *dl*-α-ionone. Helv. Chim. Acta 30: 769–774, 1947.
77. Ohloff, G., Becker, J. and Schulte-Elte, K. H.: Synthese von Exalton und racemischem Muscon aus Cyclododecanon. Helv. Chim. Acta 50: 705–708, 1967.
78. Ottoson, D.: Analysis of the electrical activity of the olfactory epithelium. Acta Physiol. Scand. 35 (suppl. 122): 1–83, 1956.
79. Ottoson, D.: Studies on the relationship between olfactory stimulating effectiveness and physicochemical properties of odorous compounds. Acta Physiol. Scand. 43: 167–181, 1958.
80. Ottoson, D. and Shepherd, G. M.: Experiments and concepts in olfactory physiology. *In* Progress in Brain Research, vol. 23, Sensory mechanisms, ed. by Y. Zotterman, Elsevier Publ. Co., Amsterdam, 1967.
81. Pauling, L.: Analogies between antibodies and simpler chemical substances. Chem. Eng. News 24: 1064–1065, 1946.
82. Prelog, V., Ruzicka, L., Meister, P. and Wieland, P.: Untersuchungen über den Zusammenhang zwischen Konstitution und Geruch bei Steroiden. Helv. Chim. Acta 28: 618–627, 1945.
83. Reese, T. S.: Olfactory cilia in the frog. J. Cell Biol. 25: 209–230, 1965.
84. Rienäcker, R. and Ohloff, G.: Optisch actives β-Citronellol aus(+)-oder(−)-Pinan. Angew. Chem. 73: 240, 1961.
85. Schneider, D.: Electrophysiological investigation of insect olfaction. *In* Olfaction and Taste, ed. by Y. Zotterman, Pergamon Press, London, 1963.
86. Schneider, D., Lacher, V. and Kaissling, K. E.: Die Reaktionsweise und das Reaktionsspektrum von Riechzellen bei Antheraea Pernyi (Lepidoptera, Saturniidae). Z. Vergl. Physiol. 48: 632–662, 1964.
87. Stuiver, M.: The Biophysics of Smell, Thesis, Groningen University, 1958.
88. Takagi, S. F. and Yajima, T.: Electrical activity and histological change in the degenerating olfactory epithelium. J. Gen. Physiol. 48: 559–569, 1965.
89. Theimer, E. T.: Personal communication, 1968.
90. Theimer, E. T. and Davies, J. T.: Olfaction, musk odor and molecular properties. Agr. Food Chem. 15: 6–14, 1967.

91. Troland, L. T.: The Principles of Psychophysiology, vol. 2, pp. 261–280, van Nostrand, New York, 1930.

92. Tucker, D. and Shibuya, T.: A physiologic and pharmacologic study of olfactory receptors. Cold Spring Harbor Symp. Quant. Biol. **30**: 207–215, 1965.

93. Veldstra, H.: Personal communication, 1961.

94. Wood, T. F.: Chemistry of the aromatic musks. The Givaudanian. Jan., Mar., May, June and Sept. issues, 1968.

95. Wright, R. H.: Odor and chemical constitution. Nature (London) **183**: 831, 1954.

96. Wright, R. H.: Odor and molecular vibration. Nature (London) **209**: 571–573, 1966.

97. Wright, R. H.: The musk odour. Perf. Ess. Oil Rec. **58**: 648–650, 1967.

98. Wright, R. H. and Robson, A.: Basis of odour specificity: Homologues of benzaldehyde and nitrobenzene. Nature (London) **222**: 290–292, 1969.

99. Zwaardemaker, H.: Die Physiologie des Geruchs, Wilhelm Engelmann, Leipzig, 1895.