

Molecular Structure and Functional Activity of Nerve Cells

EDITED BY

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A.I.B.S.

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Preface

IN THE PAST SEVERAL YEARS considerable progress has been made in the clarification of the problems of neural activity and of a host of ancillary physical and chemical events in living cells. Investigators in many fields from biology through chemistry, physics, mathematics, philosophy and psychology, have contributed new observations and concepts. They have drawn upon one another's knowledge and experience in attempts to refine existing experimental techniques and arrive at more accurate estimates of the properties and interrelationships of particular structures, mechanisms and processes at levels from the grossest complexity of the organism in toto to the most microscopic and sub-microscopic complexities of molecular structure and orientation.

Consequently, it was felt that there was need for some discussion of stimulus-response problems not only insofar as existing data are concerned, but more particularly in relation to whether or not present experimentation and concept are leading to better understanding of the crucial problems underlying neuronal behavior.

The existence of certain transient phenomena in nerve cells forms the basis for their physiology and confronts the investigator with certain sorts of problems which, although they are not unique to this field, are representative of the difficulties involved in relating biological function to molecular interaction. The reduction of experimental observations to a molecular level must involve, first of all, a recognition that a rather small number of molecular units are involved in the events leading to the initiation of excitation in an excitable system, and that, therefore, the possibility exists that ordinary statistical considerations may not apply.

In seeking both the proper questions and answers, one is confronted at the outset with the initial way station of reception—the input to the nervous system. The major questions asked are how, in general, physical stimuli are transduced into nervous excitation; and is the principle reaction chemical or physical? In other words, how are stimuli transformed into meaningful, information-carrying nervous messages? It should be pointed out that while there is no requirement that transduction imply some analogy with mechano-electrical devices, it is suggested that an important property which must be inherent in a receptor is some means of converting the energy incident upon it into nervous excitation. The second question, which appears to stress a somewhat unreal dichotomy, is less clear in that a distinction between biophysical and biochemical reactions is less and less obvious as the units under consideration approach molecular dimensions.

Impulses from the receptors are passed on to aggregates of neurons in the central nervous system. At this level one would like to know if any progress has

been made in our ability to explain observed properties of neurons in the aggregate on the basis of the differential biochemistry of the cells involved? Can we discuss the relationships of chemical structures to integrated activity or must new experimental paths be initiated which might be more productive than those now being pursued? It has been apparent for some time that not all cerebral neuronal groups, for example, behave similarly. At least part of the differential sensitivity of different types of neurons to drugs, anoxia and other environmental factors is related to either quantitative or qualitative differences in the sequence of metabolic reactions that are going on in the various cells. Nonetheless, present evidence forces one to the conclusion that certain underlying mechanisms of response are common to neurons in a wide variety of circumstances. The questions remain as to the role of biochemical reactions not only in these general responses, but also in the response mechanisms by which groups of neurons communicate with one another and do permit the organisms to be aware and well informed.

There remains for this symposium one further area which is somewhat more specific—namely, consideration of the physiological membrane. Any understanding of the various processes involved in nervous excitation devolves ultimately upon a model of some sort that represents a membrane. What is the nature of the membrane alteration that permits excitation or depression? In other words, how is molecular orientation in the membrane related to alterations in activity? In some respects this question differs from the others in that it is unlikely that the actual events occurring in the membrane can be determined directly by experimental means; rather, our understanding of such events appears more likely to be deduced from a series of models of greater and greater refinement, incorporating more and more of the phenomena actually observed. Whether there is any possibility of developing a model at present is a question that surely deserves to be examined. In addition, can any statement be made concerning the interrelationships of membrane structure and properties to physical and chemical events within the neuron? These are the fundamental mechanical questions which must be answered before understanding can be reached of the special faculties of neural tissue to receive and store information which can be communicated to and used by the organism in the proper time.

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I

RECEPTORS

Chemoreceptor Mechanisms

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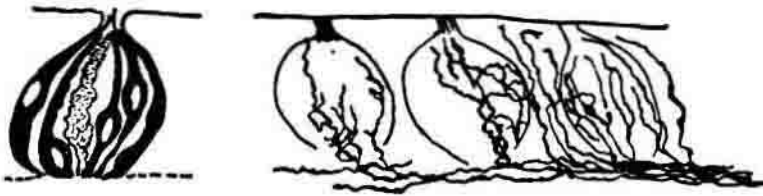
Introduction

THE FOLLOWING TWO QUESTIONS, which were propounded by the originators of this symposium, represent the starting point and the ultimate goal of much of the research which involves stimulating sensitive tissue: How are physical stimuli transduced into nervous excitation? Is the ultimate reaction biochemical or biophysical? Insofar as stimulation by chemicals is concerned there is no tissue more appropriate for investigation than the tissue which is specialized by nature to receive chemical stimuli which play an indispensable role in the daily economy of living, in short, the chemoreceptors. In our ignorance it is still expedient to refer to the principal chemical senses as taste and olfaction. The following discussion will utilize this distinction; and, since greater attention has been bestowed by experimenters on the sense of taste, the major emphasis here will be placed on that sense. The discussion will, moreover, attempt to adduce from experiments conducted on the two most extensively studied groups of animals, mammals and insects, evidence for a general theory of action of chemical stimuli.

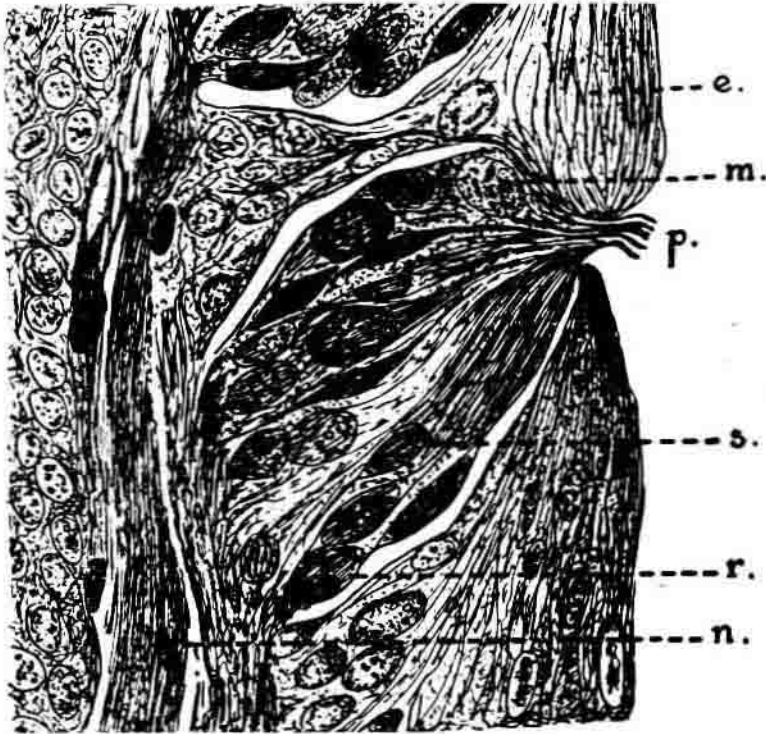
Taste

Mammals

Taste in mammals is subserved by cells within papillae situated principally on the tongue. Within the papillae the receptors are gathered together in groups, the taste buds. Within the buds lie a variable number of cells (Fig. 1). In man it is generally believed that there are four primary taste modalities, sour, salt, bitter, and sweet, and that most other taste sensations are compounded of these. The earliest suggestion that there might be specific receptors for the separate primary tastes derived from the observation that different areas of the tongue were differentially sensitive to the four qualities. Topical application of solutions



SECTIONS THROUGH TASTE-BUDS OF THE RABBIT, PREPARED BY GOLGI METHOD
Left (after von Lenhossék), showing sensory cells and a "sustentive" element;
right (after Retzius), showing terminal fibers.



SECTION THROUGH ONE OF THE TASTE-BUDS OF A PAPILLA FOLIATA OF THE RABBIT
e, superficial epithelial cells; *m*, leucocyte containing granules; *p*, gustatory pore;
s, gustatory cell; *r*, sustentacular cell; *n*, nerve fibers.
(From Quain, after Ranvier, copied in Howell.)

FIG. 1. Taste bud in the tongue of a rabbit (from Crozier, 1934)

then revealed that a single papilla might respond to one taste, all tastes, no taste, or a combination of tastes. It was not, however, until electrophysiological techniques were developed that it became at all possible to determine to what extent each single receptor was sensitive to the hundreds of compounds which are adequate stimuli for the tongue as a whole. And even now the situation is far from clear, because a single fiber may innervate more than one receptor cell. Where a fiber is seen to respond to either of two substances placed on the tongue,

TABLE 1

Scheme illustrating possible relationship between afferent impulse pattern and evoked taste sensation in the cat (Cohen, Hagiwara, and Zotterman, 1955)

Stimulus	Water Fiber	Salt Fiber	Acid Fiber	Quinine Fiber	Sensation Evoked
H ₂ O (salt 0.03 M)	+	0	0	0	water
NaCl (0.05 M)	0	+	0	0	salt
HCl (pH 2.5)	+	+	+	0	sour
Quinine	+	0	0	+	bitter

there remains a question of whether two specific receptors are involved or a single less specific one. Pfaffmann (1941) first demonstrated by recording from single fibers in the chorda tympani nerve of the cat that there were at least three physiologically different types of receptors (the term "receptor-unit" as applied by Beidler, Fishman, and Hardiman (1955) is preferable). One type responded only to acids, a second to acids and salts, and a third to acids and quinine. No fibers were then found which responded when sugars were applied to the tongue. Subsequently, "sweet" fibers were demonstrated (Beidler, 1952); and recently Cohen, Hagiwara, and Zotterman (1955) further characterized four physiologically different fibers. Their findings are summarized in Table 1.

In the frog, Zotterman (1949, 1950) and Andersson and Zotterman (1950) found some fibers which responded specifically to water and to dilute NaCl (<0.025%), some which were specific for acids, and others which responded to hypertonic NaCl and CaCl₂. In the chorda tympani of the dog, Andersson et al. (1950) detected fibers which were specific for sugars. In the rat, Beidler (1953) found fibers which responded specifically to salt, to salt and acid, and to sugar.

The foregoing results clearly indicate that there is a degree of true specificity in taste receptors of mammals and that there exists at the peripheral level a basis for the four modalities. There is evidence, furthermore, that the sensitivity of the several receptors represents a specialization of these cells and is not merely a consequence of exposure and opportunity. Beidler (1953) has shown, for example, that salt receptors in the rat have a lower threshold for NaCl (0.002 M) than other receptors innervated by the lingual nerve (+1.0 M) and than bare axon.

Granting, therefore, that there may be found in the mammal taste receptor-units which are specifically sensitive to acid, salt, acid and salt, acid and quinine, water, and sugar, what further information can be adduced concerning the characteristics of these units? Most of the experimental work which has been done has involved stimulation of large populations of receptors, i.e., areas of the tongue or individual papillae. Some of the electrical recording has been con-

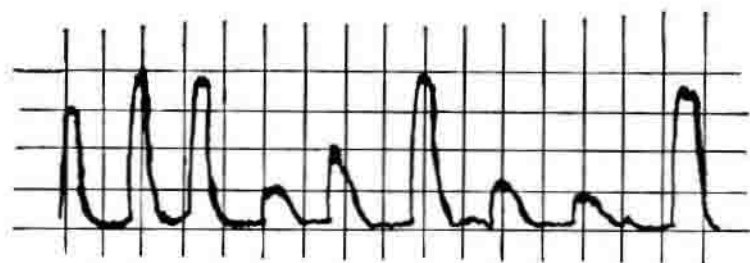


FIG. 2. Integrated electrical response of chorda tympani to various 0.1 M salt solutions flowed over tongue of rat: NH_4Cl , LiCl , NaCl , KCl , CaCl_2 , NaCl , RbCl , CsCl , NaCl . Test solutions are interspaced with water rinses. Time scale: 1 large division = 20 sec. (Redrawn from Beidler, 1953).

cerned with few-fiber preparations or with total-fiber integrated response (Fig. 2) (Beidler, 1953). Information derived from these approaches has revealed facts of the following sort.

There is general agreement insofar as the acid taste is concerned that an important constituent of the stimulus is the hydrogen ion. However, if this ion were the sole factor, all acids would be expected to taste equally sour at the same pH, and this is not so. Some workers feel that the discrepancy is to be accounted for on the basis of interaction with saliva; others do not agree (Pfaffmann, 1951, Beidler, 1952). It is clear that there is no simple correlation with pH, and, by analogy with the salts, it is not at all unlikely that the anion may have a contributory effect. In the case of insects, where there exist acid receptors unencumbered with saliva, there is again no simple correlation with pH (Chadwick and Dethier, 1947). Furthermore, as Beidler (1952) has pointed out, experiments with buffered solutions, designed to isolate the effect of the hydrogen ion, are confounded by the existence of receptors which are sensitive to salts as well as to acid.

Practically all salts are stimulating. Their stimulating efficiency in a variety of animals has been expressed as series of values based upon threshold measurements, saltiness of taste, etc. There are serial differences from one species of animal to the next and differences depending upon the criterion of response which is chosen. With regard to species differences, Beidler, Fishman, and Hardiman (1955) have reported the results of recording electrically the response of the chorda tympani to chemical stimulation of the tongue of rats, guinea pigs, rabbits, cats, dogs, hamsters, and racoons. Marked differences exist in the ability of the taste receptor-units (= a group of cells innervated by one single chorda tympani fiber) to respond to a number of substances. The rodents respond well to NaCl compared to KCl , whereas the opposite is true with the carnivores. Animals of both orders respond well to NH_4Cl . The hamster and guinea pig are easily stimulated by sucrose, whereas the cat is not.

For many species the cations can be arranged in increasing order of stimulat-

ing effectiveness as follows: $\text{Li} < \text{Na} < \text{Ca} < \text{K} < \text{NH}_4$. The anions are frequently arranged: $\text{SO}_4 > \text{Cl} > \text{Br} > \text{I} > \text{HCO}_3 > \text{NO}_3$. It is probable that both the cations and the anions play a role in stimulating the receptor. The order of efficiency of the cations as given above is also that of the ionic mobilities and the partition coefficients as well. The arrangement of the anions is less clear. The activity of the divalent cations is anomalous in that they do not fit into any of these series. The work of Beidler with the rat has shown that the divalent salts are different from the monovalent salts in several respects. Hodgson (1951) has also noticed this difference in tests with the water beetle, *Laccophilus*. In man many of the divalent salts are bitter. Moreover, each salt has a different and recognizable taste and so must stimulate different receptors or combinations of receptors.

Hardly any experiments have been undertaken to test the effect of simple organic compounds on the taste receptors of mammals. The lack may stem in part from complications introduced by the concomitant olfactory stimulation effected by most of the compounds in this category. However, data which exist for the homologous aliphatic alcohols and glycols (Figs. 3 and 4) indicate that the thresholds (molar) decrease logarithmically with increasing chain length (Renqvist, 1920; Dethier, 1951b; Gavaudan, 1948, 1951; Poussel, 1951). In the case of organic salts, Beidler (1954) found that there was a decrease in effect with chain length; however, he tested sodium salts only up to butyrate; and data of Dethier (Fig. 5) with longer series of sodium, lithium, and barium salts acting on insects show that beginning with the five carbon compound there is a logarithmic decrease similar in all respects to that observed with non-polar homologues.

The outstanding feature of all of the data accumulated from tests with sweet and bitter substances has been the remarkable nonconformity of the physiological effect with any single chemical or physical property of the sort recorded in whole or in part for the non-sweet compounds. On the contrary, there is to be noticed a much more precise dependence upon specific chemical configurations. This dependence is true of the bitter as well as the sweet substances. For example, *ortho*-tolylurea is tasteless, *meta*-tolylurea is bitter, and *para*-tolylurea is sweetish (Beidler, 1952). Other equally small changes in molecular structure are reflected as pronounced physiological changes. Stereo isomers also have different tastes. For example, D-asparagine is sweet; L-asparagine is tasteless. In the case of insects, D-arabinose is nearly five times more stimulating than L-arabinose (Hassett, Dethier, and Gans, 1950).

Insects

All of the information summarized in the foregoing paragraphs can be multiplied and elaborated a hundredfold. It is well to remember, however, that in

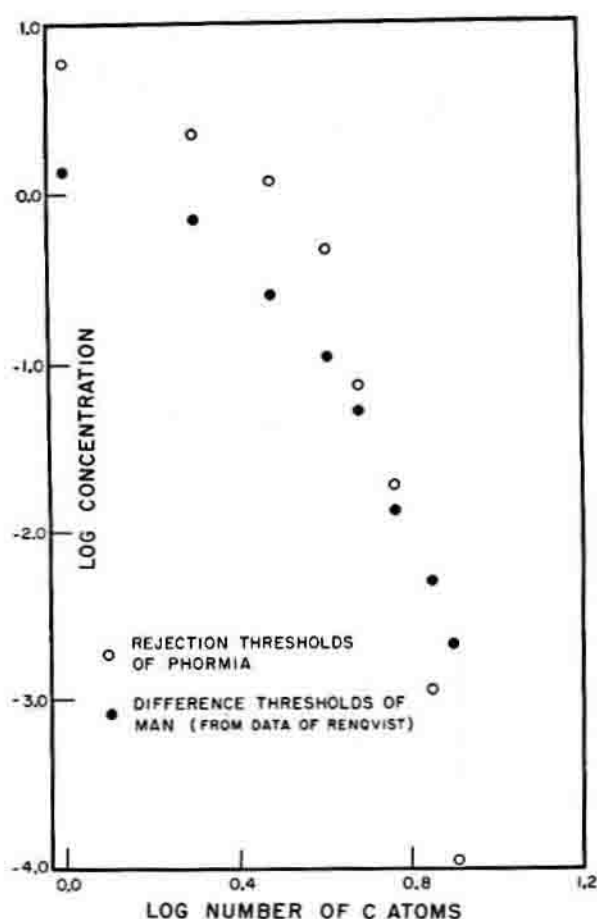


FIG. 3. Comparison of relative effectiveness of homologous alcohols in stimulating tongue of man and tarsal receptors of the blowfly (Dethier, 1951b).

all but a very few cases the information was derived from stimulation of populations of mixed receptors, and to what degree the information can be extrapolated to single receptors remains to be seen. Until very recently the situation in insects was the same. A great deal of information had been amassed as a result of stimulating receptor fields. Just as the criteria with mammals were subjective or behavioral, so in insects they were behavioral. In general the picture which emerged was one of two taste modalities, acceptable and unacceptable. (Some experiments in progress now suggest that this conclusion may be an over-simplification.) Stimulation of insect receptors by electrolytes (Frings, 1946, 1948; Frings and O'Neal, 1946; Hodgson, 1951) revealed that, with minor exceptions, the stimulating efficiencies of cations and anions follow the pattern already given. Furthermore, there was evidence that both ionic species are contributing to the event. The similarity of acid stimulation in insects to that in man has already been mentioned. In the case of sugars the high degree of molecular specificity already noted with man is found to exist in insects in as high or possibly in a higher degree (von Frisch, 1935; Hassett, Dethier, and Gans, 1950; Dethier, 1955). Data relative to the stimulating effects of organic compounds are far more complete for insects than for man (Dethier, 1953). Here also there is a

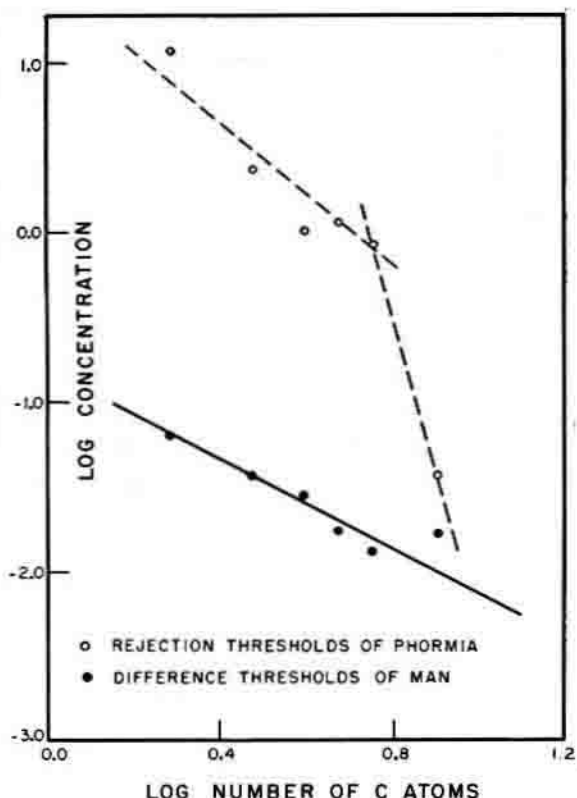


FIG. 4. Comparison of relative effectiveness of homologous glycols in stimulating tongue of man and tarsal receptors of the blowfly (Dethier, 1951b).

general similarity to the results obtained with man in that the threshold decreases logarithmically with increase in chain length. For insects this is not an even decrease. In the case of homologous alcohols the curve shows a break in the region of the four and five carbon compound (Fig. 6). In series of aldehydes and ketones the break is in the region of the three carbon homologue (Fig. 7). In the glycol series there is a break in the region of the six carbon compound. When large chain polymers are employed as stimuli, the break persists but in the opposite direction, i.e., the increase of stimulating effect with increasing chain length is less pronounced for the higher members than for the lower ones (Fig. 6). In short, polymer mixtures do not react the same way as pure compounds.

It is possible to relate threshold rather accurately to chain length and to make precise predictions on the basis of these relationships. But of all the colligative properties of homologous compounds the only one which permits bringing members of all series into one frame of reference is solubility. The threshold is directly proportional to the water solubility (Fig. 8) (Dethier and Chadwick, 1950). Additional evidence that solubility is of importance in this connection has been presented by the work of Dethier (1951a), which shows that the measured thresholds of alcohols are altered as the alcohol is presented as an aqueous solution, a glycol solution, a mineral oil solution, (Fig. 9). When threshold values

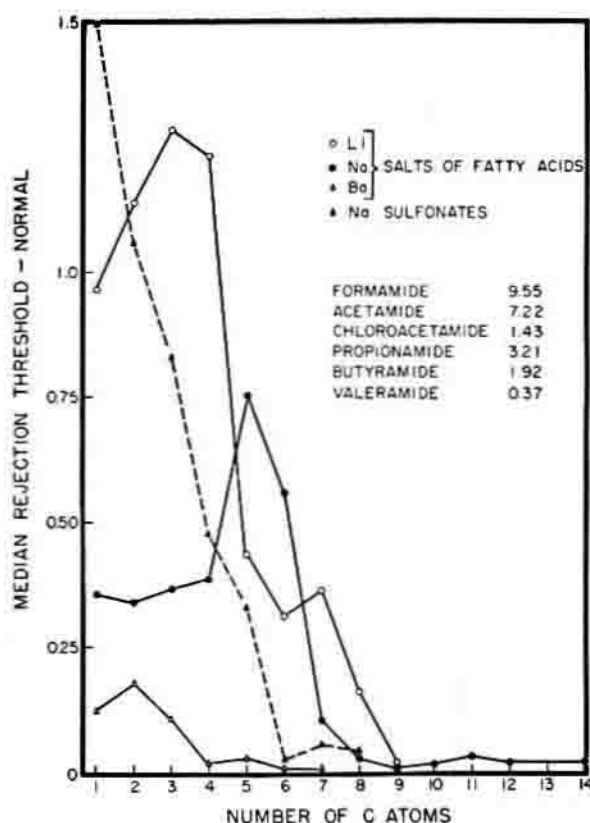


FIG. 5. Relation between the stimulating efficiency of organic salts and the chain length of the anion.

are expressed as thermodynamic activities rather than as moles, the differences between successive homologues of a series are not so marked, but a plot of the log of these values against the log of activity coefficients (Fig. 10) does not produce a straight line of the sort that one is accustomed to expect from parallel experiments on narcosis (for a complete discussion consult Ferguson, 1951; Brink and Posternak, 1948; Dethier, 1954b).

An attempt was made to obtain with human beings some data comparable to those just outlined above even though the statement is frequently made that man tastes only those substances which are in aqueous solution. Tests revealed that it was possible to measure taste thresholds for alcohols dissolved in mineral oil (Dethier, 1952). As was the case with insects, man's thresholds for the homologous alcohols in oil are nearly but not exactly identical for each homologue. A plot of the oil threshold/water threshold ratio against the activity coefficient (Fig. 11) indicates that the members seem to fall into two groups, the higher members being in one group and the lower in another. If the threshold ratios are plotted against the oil/water partition coefficients, it can be seen that the two sets of values are not the same (Fig. 12). This lack of complete agreement may be attributed either to the interference of saliva and to the fact that one is working with large populations of mixed receptors, or it may indicate that oil/water partitioning is not the limiting factor.

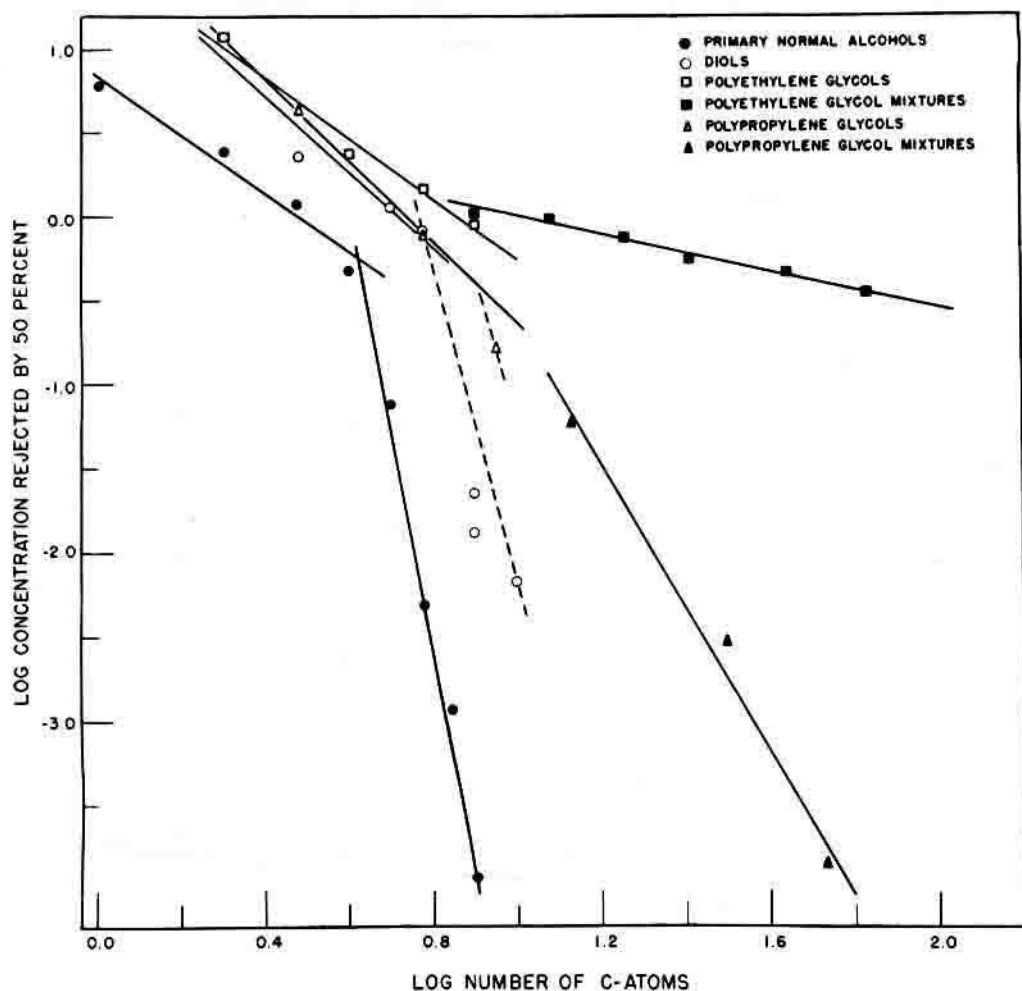


FIG. 6. The relation between the rejection thresholds of alcohols and glycols by the blowfly and the chain length (Dethier and Chadwick, 1948b).

With insects one tremendous advantage has recently been granted us. It is now possible in the blowfly, *Phormia regina*, to work behaviorally and electrophysiologically with a single-receptor preparation. The histology of the receptors has been studied in great detail (Dethier, 1955). It is possible to stimulate a single receptor and elicit a complete normal behavioral response (Dethier, 1955) and a clear electrical response (Hodgson, Lettvin, and Roeder, 1955). The receptor organ concerned is analogous to a single mammalian taste bud in that it contains more than one receptor. Two receptors have been demonstrated, but each is innervated by its own nerve fiber and each can be stimulated singly. The receptor organ is a hair (sensillum trichodeum) containing three bipolar neurons two of which send distal processes to the tips (Figs. 13 and 14) where they are clearly invested with a cuticular covering which possesses, however, permeability characteristics radically different from those of the cuticle on the rest of the hair. Furthermore, the cuticle at this point has a very low electrical resistance as compared to the rest of the hair which is nearly perfectly insulated. The process of the third neuron ends at the base of the hair and may not

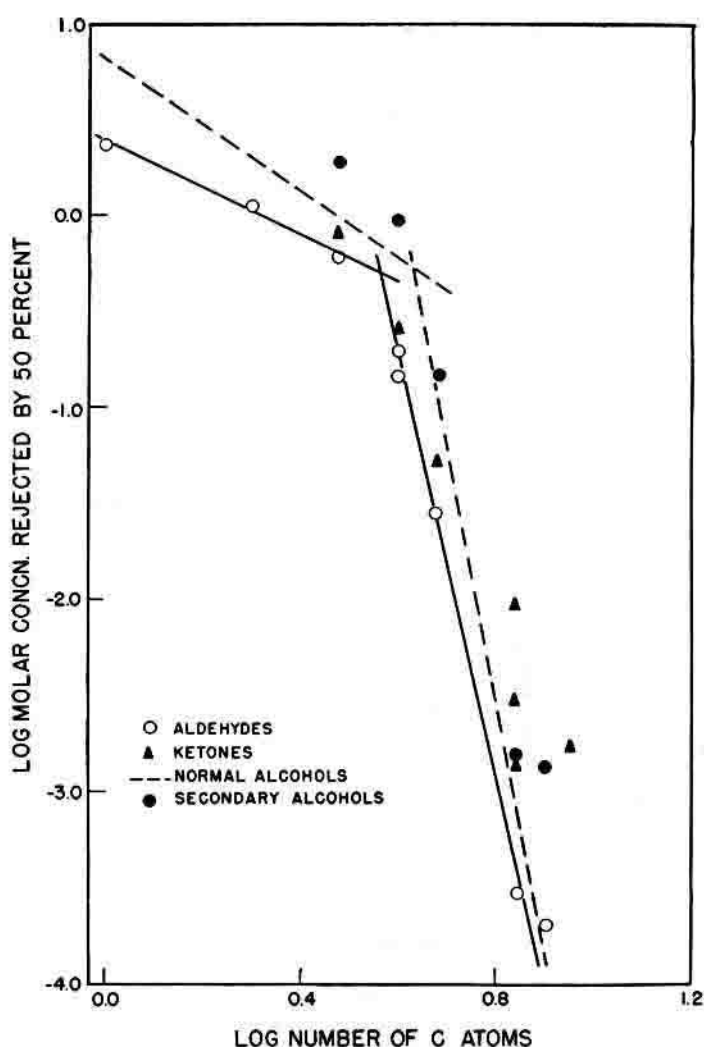


FIG. 7. The relation between the rejection thresholds of alcohols, ketones, and aldehydes by the blowfly and the chain length (Chadwick and Dethier, 1949).

be concerned directly with chemoreception, although this remains to be proven. The proximal processes of all three cells travel directly to the central nervous system without, as far as we know, synapsing. Here, then, is a primary neuron, one end of which is exposed to the stimulus and the other end of which makes its first connection deep within the central nervous system. This is indeed a convenient preparation to have at hand.

Only the tip of the hair, where the two fibers terminate in a minute papilla (Fig. 14), is sensitive to stimulation. A single hair is sensitive to every compound to which the animal as a whole is responsive provided that a threshold concentration can be applied. Stimulation by certain sugars elicits a behavioral response in the nature of a proboscis extension. Stimulation by non-sugar compounds elicits withdrawal of the proboscis; or it inhibits extension. A variety of tests, such as simultaneous stimulation of one hair with sugar and another with salt, show that the inhibiting compounds are truly stimulating. The elec-